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P. J. du TOIT,
Director of Veterinary Services.

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D. G. Steyn	The Effects of Sulphur on Merino sheep.

17th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1931.

Studies in Mineral Metabolism, XIV.

Inorganic Phosphorus in the Blood of Pregnant Heifers.

By A. I. MALAN, D.Sc., Biochemist, Onderstepoort, and J. G. BEKKER, B.Sc.Agric., B.V.Sc., Veterinary Research Officer, Vryburg.

Since phosphorus deficiency causes a drop in the inorganic phosphorus fraction of the blood of bovines (Malan, Green and Du Toit, 1928), and since an attempt is being made to establish which areas are phosphorus deficient in South Africa by means of soil, pasture and mainly blood analyses (Malan, 1930), work on the variations of the inorganic phosphorus in the blood of bovines under farming conditions is being undertaken. In brief outline the mineral survey work includes the analysis of blood samples forwarded by field veterinarians to Onderstepoort. These blood samples are taken from cattle in areas where both soil and vegetation are collected and forwarded to Onderstepoort for the determination of their inorganic constituents. It is clear that the blood so obtained from the field has been drawn from practically all classes of stock and the interpretation of the results is a difficult matter unless some standard of comparison is available. Climatic conditions influence the phosphorus content of natural pasture which in its turn is perhaps the main factor that determines the inorganic phosphorus content in the blood of bovines on such pasture. Then too, the question of age, pregnancy, lactation and growth is important and undoubtedly influence the inorganic phosphorus content of the blood of cattle. Hence their effect upon blood phosphorus had to be studied in order to obtain data for comparing the results of the blood analyses in the mineral survey work.

Experimental Work.

The breeding experiment described by Theiler, Green and Du Toit (1927), and reported on by Du Toit and Bisschop (1929), provided excellent material for this type of work. The additional advantage existed in that both phosphorus deficiency and sufficiency under practical farming conditions could be studied in relation to their effect upon the blood phosphorus. Four breeds of cattle of practically all ages were available. The present investigation, however, began with twenty pregnant heifers, and was carried over a period of twelve

months. An elaborate scheme which will include cattle of all ages from the four available breeds and of which the blood phosphorus will be done at regular intervals for a year, is under consideration. Hence this report must be regarded as preliminary only and as the beginning of a larger plan.

The inorganic phosphorus was determined by the method described by Green (1928) with the slight modification that the filtrates were not neutralised before adding the reagents. This was found necessary as no detectable change in the readings was observed, whether the solutions had been neutralised or not.

Table 1 gives the inorganic phosphorus content of the blood of heifers determined at monthly intervals and weekly determinations of their calves from birth onwards until the age of 2 months.

Discussion of Results.

Although the results given in Table I show no extraordinary characteristic, they are interesting and many points are noteworthy.

First of all and perhaps most striking is, that for the entire period it would have been an easy matter to distinguish according to blood analyses control animals from those receiving bonemeal. Slight variations, in some cases quite marked, are noticeable for individual animals. This is especially true for the values obtained during September and October, 1929. That incidentally was the period during which the first spring rains fell and green succulent grass, therefore, became fairly abundant.

The inorganic phosphorus of the blood increases as would be anticipated with increase of green grass; in other words, a larger intake of phosphorus in the feed is reflected as a higher inorganic phosphorus in the blood of the animal. Further study of the blood phosphorus during the period of abundance of new growth may reveal the fact that animals on natural pasture during that period receive sufficient phosphorus in the feed and do not require a phosphatic supplement. The figures for inorganic phosphorus of the blood of control animals taken as a whole still indicate a definite deficiency of phosphorus in the feed during September and October, but then the systems of these animals had to adapt themselves to the low phosphorus in the feed prior to the period of abundance, resulting most probably in a depletion of phosphorus reserves.

It would, therefore, be unnatural for such animals to build up their systems to a state of phosphorus sufficiency in the same comparatively short period that animals which had been on a bonemeal ration would.

The inorganic phosphorus of the blood is apparently so closely associated with the nutritional aspect that it would be unwise to mention anything about the effect of pregnancy from the available figures beyond the observation that a drop occurs gradually as the gestation period approaches termination. This is interesting in view of the fact recorded by Du Toit and Bisschop (1930) that the calves of both control cows and those receiving a daily ration of bonemeal do not show notable differences in weight at birth. The drop in inorganic

phosphorus is more noticeable in the blood of the control heifers than in that of the bonemeal group. Apparently this is only further proof that nature has made provision to prevent the birth of weaklings, as far as the mineral constituents of the body are concerned. As the gestation period progresses the demand for phosphorus by the foetus on the mother becomes greater and this evidently causes a further drop in the blood phosphorus of the mother unless the phosphorus in the feed be increased accordingly. This decrease is intensified when lactation begins, and falls in some cases to less than 1 mg. per 100 c.c. Such readings have been taken as 1 mg. for colorimetric matching below that limit is questionable. The problem of milk production, in view of the fact that the total phosphorus in the milk of control cows is not subject to greater variation than that in the milk of cows receiving bonemeal, apparently becomes a vital one for the control animal. Milk yield decreases very rapidly, and the dam loses condition to such an extent that she becomes superficially indistinguishable from a scrub. In most cases the dams suffer from styfsiekte so badly that they can hardly walk, craving increases and the animal looks pitiable. The calf becomes stunted in growth, but is by no means such a neglected object as its dam. This is the type of animal that shows approximately 1 mg. inorganic phosphorus in its blood.

A glance at the analyses of calf blood also given in Table I for convenience, indicates that the inorganic phosphorus fraction in the blood of calves at birth is appreciably higher than that of the mother, rises sharply and is still high after eight weeks. It does not seem impossible, however, that the blood of a calf at birth could agree in inorganic phosphorus with that of its mother, as noted by Green and Macaskill (1928), when one remembers that blood phosphorus is most easily affected by the phosphorus content of the feed. An interesting little study would be regular blood analyses for inorganic phosphorus of an animal on excess available phosphorus during pregnancy and lactation. At all events without further work it would be dangerous to theorise on the possible significance of the inorganic phosphorus fraction of the blood of calves at birth when compared with that in the blood of the dam. Normally this fraction could very easily be increased in the blood of bovines by giving or withholding phosphorus in the feed so that, superficially, it may be made to approach or vary from the inorganic phosphorus fraction of the blood of the calf, which is apparently more constant and not influenced by the intake of phosphorus of the dam. In Table I, the average figures for inorganic phosphorus in the blood of calves at birth of control mothers and of those receiving bonemeal are 5.2 and 5.1 mg. respectively. In view of this it would be interesting to find out whether the inorganic phosphorus content of the blood of calves at birth can be affected at all by an increase or a decrease in the phosphorus content of the food of the dam. From the available figures this does not seem to be the case. The rise in inorganic phosphorus in the blood of calves is in all cases quite definite, and apparently begins soon after birth.

It may be noted in passing that heifer No. 1850 had no calf and that the inorganic phosphorus content of her blood remained comparatively high.

FIGURE I.

INORGANIC PHOSPHORUS IN MGM. PER 100 C.C. BLOOD.

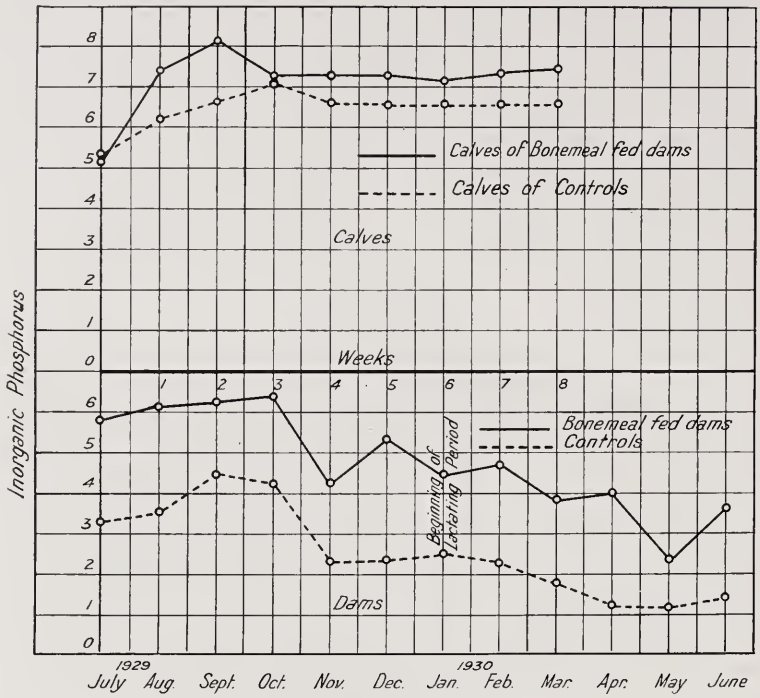

Figure I gives a graphical summary of the data tabulated in Table I. On the whole the average figure for inorganic phosphorus in the blood of the calves of the bonemeal fed mothers is slightly, but apparently definitely higher, than that in the blood of the calves of control mothers. This is probably due to the increased milk yield of the cows receiving bonemeal whose calves would therefore get more phophorus than their less fortunate brothers and sisters belonging to control cows.

From the limited data available it does not seem that the phosphorus content of the blood of one breed of cattle varies from that of the other. However, this point will be brought out more clearly in the larger experiment which is about to be undertaken.

SUMMARY.

1. The inorganic phosphorus in the blood of pregnant heifers has been determined through the greater part of the gestation period and for two months after calving, a period of twelve months in all.

2. The control heifers showed low figures for inorganic phosphorus in their blood when compared with heifers receiving bonemeal.

3. It is difficult to say from the limited data whether gestation has any effect on the inorganic phosphorus of the blood.

4. The inorganic phosphorus in the blood of the calves has also been done weekly from birth up to the age of eight weeks.

5. Most of the calves showed a low figure for inorganic phosphorus in their blood at birth. These low values increased very rapidly sometimes, and remained definitely higher than those for the inorganic phosphorus in the blood of the dams until the experiment was discontinued when the calves were two months old.

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TABLE I.

INORGANIC PHOSPHORUS DURING PREGNANCY AND LACTATION. INORGANIC PHOSPHORUS IN THE BLOOD OF CALVES DURING THE FIRST TWO MONTHS OF LIFE.

(C=controls; B.M.=animals receiving bonemeal; I.P.=Inorganic phosphorus per 100 c.c. blood).

No.	Breed.	Age. Years.	When Served.		Monthly Determination of I.P. from 3/7/29–27/5/30.												Date of Birth of Calf.	I.P. at Birth.	Weekly Determination of I.P. from Birth until 8th Week.							
					July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May.	June.			1.	2.	3.	4.	5.	6.	7.	8.
1908	Half-bred Fries.	3	14/5/29	BM	6·4	6·9	6·8	6·7	4·2	5·2	4·5	4·5	2·5	3·7	1·6	2·9	27/2/30	4·4	7·2	6·9	7·0	7·4	6·8	6·4	6·9	6·8
2001	,,	3	6/5/29	BM	5·5	6·5	6·2	6·6	5·1	4·7	4·9	5·0	3·1	4·1	2·0	4·3	18/2/30	4·3	5·8	6·6	6·7	6·7	7·6	6·2	6·5	6·2
2341	,,	2	7/4/29	BM	6·0	6·4	6·6	7·3	5·3	6·2	4·4	4·8	3·7	4·5	3·0	4·3	11/1/30	4·0	9·8	9·9	7·7	6·7	7·1	7·1	7·1	6·2
2335	Half-bred Afrikander	2	27/4/29	BM	6·6	5·7	6·8	7·5	4·0	6·2	4·8	4·7	4·1	4·1	2·8	4·3	4/2/30	5·8	5·7	7·7	7·4	9·4	8·2	8·1	7·1	7·8
2338	,,	2	4/4/29	BM	4·6	6·2	6·8	6·6	2·7	5·5	4·3	6·0	4·8	4·0	1·9	2·8	15/1/30	5·7	8·4	7·5	7·5	7·0	7·3	7·6	7·1	8·3
2347	,,	2	5/4/29	BM	6·5	6·2	6·0	6·7	4·9	5·2	4·1	5·4	3·4	2·9	2·2	3·4	9/1/30	6·5	8·7	8·5	7·4	7·5	7·7	7·6	8·0	7·2
2275	Half-bred Sussex	2	5/4/29	BM	6·3	5·7	6·8	6·1	4·0	4·8	4·7	4·2	4·8	4·8	2·9	4·7	8/1/30	—	7·0	8·7	8·7	7·1	6·8	6·7	7·6	7·6
2343	,,	2	9/4/29	BM	6·1	6·2	5·3	6·1	4·7	6·4	4·8	4·3	4·0	4·1	2·2	3·8	14/1/30	4·8	6·8	8·7	7·2	6·6	7·0	6·7	7·7	8·0
2019	Half-bred Red Poll	3	2/6/29	BM	5·3	5·6	5·4	6·2	4·2	4·3	4·3	5·0	3·9	3·8	2·4	3·2	4/4/30	Calf died.								
	AVERAGES	—	—	—	5·9	6·1	6·3	6·6	4·3	5·4	4·5	4·9	3·8	4·0	2·3	3·7	—	5·1	7·4	8·1	7·4	7·3	7·3	7·1	7·3	7·3
1899	Half-bred Fries.	3	14/5/29	C	2·3	2·3	2·9	3·4	1·3	1·2	1·9	2·7	—	1·2	0·9	1·2	8/2/30	4·9	5·8	6·1	7·1	6·8	6·8	6·8	6·8	7·2
1981	,,	3	7/4/29	C	2·7	3·2	2·8	3·5	1·2	0·9	1·1	1·0	1·0	1·0	1·0	1·4	13/1/30	4·7	6·0	7·7	7·7	6·6	6·3	6·7	6·2	6·7
2286	,,	2	16/4/29	C	2·7	3·4	3·6	4·0	1·8	1·9	2·2	2·4	2·0	1·1	1·9	2·5	14/1/30	Calf died.								
1962	Half-bred Africander	3	1/6/29	C	3·6	3·7	6·1	5·0	2·4	2·1	2·1	1·6	2·2	1·0	1·0	1·3	11/3/30	5·4	6·7	7·7	7·2	6·8	6·6	7·1	6·2	7·2
1989	,,	3	27/4/29	C	2·7	2·9	4·2	4·2	2·3	1·9	2·4	2·9	2·3	1·0	1·0	1·2	22/2/30	6·5	6·9	7·2	8·0	6·4	5·9	6·2	7·2	6·2
2378	,,	2	18/4/29	C	3·5	4·4	5·6	5·1	3·6	2·8	3·1	2·2	2·9	Died.			4/2/30	4·3	6·3	7·8	8·1	7·7	8·0	7·6	7·2	6·1
2399	,,	2	25/4/29	C	3·5	5·0	5·5	5·4	3·9	3·1	3·0	2·2	1·2	1·0	1·0	1·3	9/2/30	5·2	7·3	6·6	7·5	6·7	7·5	6·6	6·4	7·0
2400	Half-bred Sussex	2	30/4/29	C	4·3	5·1	4·9	5·2	2·3	2·2	2·6	2·4	2·2	1·5	1·1	2·1	4/2/30	5·9	6·5	6·7	6·9	7·0	7·0	6·2	5·5	5·9
2371	,,	2	5/4/29	C	4·4	4·6	4·7	4·3	2·7	3·1	1·9	2·7	1·6	1·0	1·0	1·3	20/1/30	5·4	6·0	6·0	7·0	6·7	6·6	7·7	7·5	6·9
2025	Half-bred Red Poll	3	22/4/29	C	3·5	3·7	4·2	4·2	1·6	2·1	4·2	2·4	2·4	2·1	1·3	1·7	30/1/30	5·3	5·8	6·2	5·4	6·2	5·6	6·6	7·3	5·9
	AVERAGES	—	—	—	3·3	3·7	4·5	4·3	2·3	2·2	2·5	2·2	1·8	1·2	1·1	1·5	—	5·2	6·3	6·8	7·2	6·7	6·7	6·8	6·7	6·6
1850	Half-bred Africander	3	22/6/29	BM	5·8	6·8	7·4	6·3	4·9	5·2	4·3	4·8	4·0	4·0	3·9	Not pregnant.										

Studies in Mineral Metabolism, XV.

Potassium Iodide in Poultry Farming.

By A. I. MALAN, D.Sc., Biochemist, Onderstepoort.

Although giving licks containing iodine or sometimes iodised salt has become almost universal practice amongst stockmen in many parts of the world, it is strange how little evidence can be found from properly controlled experiments for believing that iodine has beneficial effects in nutrition. As a matter of fact, negative results, or even cases which tend to show that iodine administration in small quantities produces toxic effects are just as abundant. It must be understood that the above does not refer to work done on goitrous conditions, for feeding iodine supplements under such circumstances certainly seems justifiable.

No attempt will be made to go into the vast amount of literature on iodine metabolism, nor will it be necessary for the consideration of the results that are given in this paper. It may be mentioned, however, that hardly any reference to the effect of iodine feeding on poultry could be found in the available literature. Evvard (1929) worked on swine and sheep mainly, whereas Orr and Leitch (1929) in their excellent review article "Iodine in Nutrition" give no references to work done on poultry either by themselves or by other investigators.

The present investigation was undertaken to obtain reliable data on the effect of iodine, given as potassium iodine in the mash of poultry, on the health, egg production and the fertility of the eggs of hens. Pure-bred white leghorn hens were available and the first experiment was conducted for the period July-October, 1929.

Table I gives a summary of the egg production and the mortality of the hens. The experiment was done in duplicate as indicated.

TABLE I.

Date.	Mineral Mixture + KI.		Mineral Mixture — KI.		KI only.	
	Group 1.	Group 2.	Group 1.	Group 2.	Group 1.	Group 2.
July.....	42	52	64	41	71	17
August.....	124	83	139	148	146	98
September.....	161	129	155	171	171	159
October.....	146	139	99	122	133	143
Totals.....	473	403	457	482	521	417
Mortality....	3	1	1	1	0	0

The supplements were fed in the dry mash to 6 groups of hens of 10 each. The groups were selected to be as nearly alike as possible by a poultry expert. The mineral mixture is that given by Corrie (1928) and 3 lb. of it were added to 100 lb. of mash.

Mineral Mixture.		*Mash*	
Bonemeal	50 lb.	Bran	65 lb.
$CaCO_3$	23 lb.	Charcoal	5 lb.
NaCl	20 lb.	Fishmeal	8 lb.
Sulphur	4 lb.	Mealiemeal	15 lb.
$FeSO_4$	3 lb.	Bonemeal	8 lb.
KI	4 oz.	Common salt	.25 lb.
		Sulphur	.5 lb.

In addition, the hens received a supply of grain at the rate of 2 oz. per bird per day.

There is obviously no significant difference in the number of eggs produced by the various groups. Still, it was regretted that two pens of control hens had not been included. It was, therefore, decided to record the egg production of two control groups as well as that of the experimental groups for another period of four months, viz., November, 1929, to February, 1930. The results are given in Table II.

TABLE II.

Egg Production and Mortality.

Date.	Minerals + KI.		Minerals − KI.		KI only.		Controls (Mash only).	
	Group 1.	Group 2.	Group 1.	Group 2.	Group 1.	Group 2.	Group 1.	Group 2.
November..	167	148	137	166	140	153	145	123
December...	157	141	130	152	132	158	159	141
January....	131	132	102	145	124	140	161	134
February...	132	120	117	129	97	89	129	124
Totals....	587	541	486	592	493	540	594	522
Mortality	2	0	1	1	1	0	0	1

As the main point of this investigation was to find out what effect iodine had on egg production when it is added to the food of poultry, it was decided to select two other groups each of 100 pullets of the same age. Give the one group the potassium iodine supplement in the mash and keep the other as controls.

A summary of the egg production of these two groups of pullets is given in Table III.

TABLE III.

Egg Production.

	KI only.	*Control pullets.*
November, 1929	1,416	1,433
December, 1929	728	899
January, 1930	1,123	1,160
February, 1930	888	1,150
Totals	4,149	4,642

The fertility of the eggs produced by hens receiving iodine and by control hens respectively, was tried during the breeding season, July-September, 1930. The hens were trap-nested and the eggs marked and incubated in a Hearson's incubator from time to time, together with other eggs in the ordinary way. The eggs of each hen in the experiment were placed in muslin bags in the incubators a few days before hatching. Six hens received potassium iodide and five were kept as controls. In this, as in the other experiments, potassium iodide was fed at the rate of 3.5 gm. per 100 lb. of mash, according to Corrie's recommendation (1928).

A summary of the results is given in Table IV.

TABLE IV.

(July-September, 1930.)

No. of Hen.	Eggs Set.	Infertile.	Dead in Shell.	KI Supplement.	Percentage Hatched.
278.....	39	6	10	+	59·0
279.....	10	0	0	+	100·0
281.....	25	4	6	+	87·0
282.....	23	1	2	+	60·0
537.....	18	2	1	+	83·4
538.....	9	3	1	+	55·6
Averages....	20·7	2·7	3·3	+	74·1
277.....	26	7	1	—	69·4
283.....	27	1	1	—	92·8
284.....	37	3	2	—	86·6
540.....	16	2	0	—	87·6
541.....	33	2	3	—	85·0
Averages....	31·8	3·0	1·4	—	84·3

Discussion of Results.

A comparison of the egg production of the groups in Table I shows no outstanding difference for any group. If the difference in the total number of eggs produced by the duplicate batches of any

group be taken as approximately a normal fluctuation, then the iodine has had neither beneficial nor detrimental effects on the egg production. This observation applies to the results given in Tables II and III as well. It may be mentioned that in only one of the nine cases was the egg production better in a group receiving potassium iodide in the mash than in the control group.

The results in Table IV are interesting, but not conclusive. It is true that the higher percentage of the eggs of the control group hatched than of the group receiving potassium iodide, but the difference without confirmation is not particularly significant.

Summary.

Figures are presented giving the egg production, mortality and fertility in a series of experiments with White Leghorn hens over periods of four months each, during 1929 and 1930. There is no reason for believing that the basal ration was iodine deficient, nor is it possible to conclude that the groups receiving a potassium iodide supplement showed an advantage in respect of egg production, mortality and fertility over the groups on the basal ration.

The author wishes to express his gratitude to Mr. G. S. G. Malan, Hanover, for his assistance and many valuable suggestions during the course of this investigation.

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Studies in Mineral Metabolism XVI.

The Micro-determination of some Inorganic Elements in Blood and Vegetation.

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The micro-determination of phosphorus, calcium, sodium, potassium, magnesium and chlorine in blood was begun at this laboratory in 1926, when blood analyses were carried out in connection with a nutrition experiment. Blood analyses and grass analyses now form quite an appreciable part of the activities in the study of nutritional problems here and the methods in vogue have been modified from time to time by various workers, with the main object of reducing the time factor while increasing the number of analyses that could be done by a limited staff. The authors claim no originality for the methods, as these have been collected and modified from the literature.

Debatable points in the procedure have been investigated by various members of the staff who found, without exception, that once micro-technique had been acquired these methods were both reliable and rapid.

A complete analysis of twelve to fourteen blood samples could be carried out fairly comfortably in a 48-hour week. More limiting factors are present in grass analyses such as the size of the electric furnace and that of the centrifuge available. With a small electric oven which holds only three platinum crucibles at the same time and a four cup centrifuge 4 to 5 analyses of grass and vegetation have been completed per week over a period of months by one chemist.

Preparation of Material for Analysis.

Blood.—Blood is drawn from the jugular vein into bottles containing the anticoagulant, viz., 1 c.c. of a 20 per cent. lithium citrate solution per 100 c.c. blood. Twenty c.c. blood are then transferred in a pipette to a flask containing 80 c.c. Trichloracetic solution (5 per cent.) The flask is closed with a rubber stopper and shaken vigorously to ensure the complete precipitation of the proteins. After 30 minutes the mass in the flask is filtered through Whatman No. 40 filter paper. The filtrate, called in this article Trichloracetic acid-filtrate, is used for the determination of the inorganic constituents in question.

Vegetation.—(*a*) Extract for the determination of K, Na, Ca and Mg.

Approximately 10 grams of finely ground grass of known moisture content are accurately weighed into a platinum crucible of known weight, and ashed in an electric muffle, just below glowing heat. After six or seven hours, whether ashing is complete or not, the crucible is allowed to cool in a dessicator. The ash is then just covered with concentrated Hydrochloric acid, which is then evaporated on a waterbath and the ashing completed in the muffle as before. After cooling 20 c.c. N HCl are added to the crucible, which is warmed on the waterbath for a few minutes and the contents are filtered quantitatively into a 100 c.c. flask through Whatman No. 40 filter paper. Washing the filter is repeated at least five times with distilled water. The flask is allowed to cool and water is added to the 100 c.c. mark. It is best to use double distilled water for the preparation of the extract.

(b) *Extract for the Determination of P and Cl.*—10 grams of vegetation are thoroughly moistened with absolute alcohol which is then evaporated on a steam bath. A few c.c. of distilled water and 10 c.c. of 10 per cent. calcium acetate solution are then added. After being dried on a steambath the contents are ashed below glowing heat in an electric muffle. When ashing is complete 10 c.c. of dilute nitric acid (1 vol. H_2O + 1 vol. conc. HNO_3) are added and the contents filtered quantitatively into a 100 c.c. flask. Water is added to the mark.

1.—Determination of Sodium.

Method: Sodium is precipitated as Uranyl-zinc-sodium acetate $(UO_2)_3ZnNa\ (CH_3COO)_9\ .9\ H_2O$ which is dissolved in acetic acid and on addition of Potassium Ferro-cyanide gives a red-brown colour, whose intensity is proportional to the amount of Uranium present.

$$K_4Fe\ (CN)_6 + UO_2(CH_3COO)_2 = UO_2K_2Fe\ (CN)_6 + 2\ CH_3COOH.$$

Sodium-free glassware only must be used.

Reagents: 1. Precipitating reagent:—

(*a*) 10 grams Uranyl acetate.
6 c.c. 30 per cent. Acetic acid made up to 50 c.c. with water.

(*b*) 30 grams Zinc acetate.
3 c.c. 30 per cent. Acetic acid made up to 50 c.c. with water.

The two solutions are mixed while still warm, allowed to stand overnight, filtered and kept in a glass stoppered bottle.

2. 20 per cent. $K_4Fe\ (CN)_6$ solution.

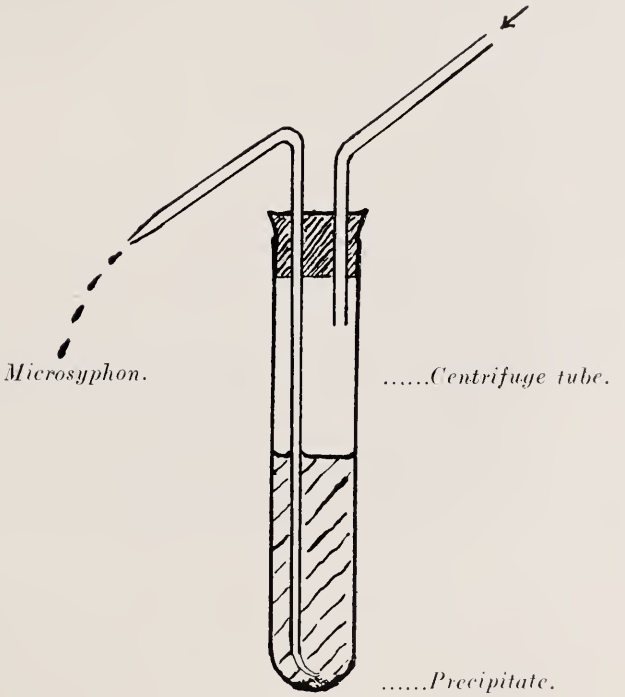
3. Absolute alcohol.

4. Saturated alcoholic zinc acetate solution.

5. Standard solution of NaCl containing .1 mgm. Na per c.c.

6. 10 per cent. acetic acid solution.

Procedure.—(*a*) For Blood: 10 c.c. Trichloracetic acid filtrate are made up to 50 c.c. with water in a measuring flask. 1 c.c. of this solution is transferred to a centrifuge tube, 3 c.c. of absolute alcohol are added and 2 c.c. of the precipitating reagent. The tube is twirled to ensure thorough mixing, closed with a rubber stopper and inverted several times. 1 c.c. Standard solution is treated similarly. After standing for at least 30 minutes the tubes are centrifuged for 5 minutes at 1,500 r.p.m. and the supernatant liquid syphoned or poured off, the procedure depending on the type of tube. The following was found very successful:—



Without disturbing the precipitate in the least 5 c.c. absolute alcohol are carefully added against the side of the tube. The tubes are centrifuged for 5 minutes and this procedure of washing is followed 3 times in all. The light yellow precipitate is treated with .5 c.c. 10 per cent. acetic acid solution and a few c.c. of water are added until the precipitate has dissolved completely. The solution is then transferred quantitatively to a measuring flask whose capacity depends on the apparent bulk of the precipitate. If the latter seems approximately twice as much as that of the standard solution it is made up to 100 c.c. and if half as much to 25 c.c. The standard is made up to 50 c.c., .25 c.c. of K_4 Fe $(CN)_6$ solution is added for every 25 c.c. solution. The flasks are inverted to mix and the colours compared in a du Bosc colorimeter after 5 minutes when they have developed fully.

Calculation when using 1 c.c. trichloracetic acid filtrate:—

$$\frac{30 \times .1 \times 100}{\text{Reading} \times .04} = \text{mgm. Na per 100 c.c. blood.}$$

(*b*) *For grass*: It is necessary to eliminate the phosphate here. 2 c.c. Extract are pipetted into a centrifuge tube and 6 c.c. alcoholic zinc acetate solution are added. The tubes are corked to prevent evaporation, inverted a few times and allowed to stand for 30 minutes. The tubes are then centrifuged for 5 minutes. The contraction in volume is such that 4 c.c. become 3.915 c.c.

Generally 5 c.c. of the supernatant liquid afford a suitable aliquot for colorimetric matching against .1 mg. sodium. The rest of the procedure is the same as that for blood.

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II.—Determination of Potassium.

Method: Potassium is precipitated as $K_2Na\,[CO(NO_2)_6]$ in which it is determined titrimetically.

Reagents: 1. Sodium cobalti nitrite solution. Precipitating Reagent:—

(*a*) 25 gm. cobalt nitrate are dissolved in 50 c.c. water and 12.5 c.c. glacial acetic acid added.

(*b*) 120 gm. sodium nitrite are dissolved in 180 c.c. water. This gives a total volume of about 220 c.c.

Solution (*a*) is added to 210 c.c. of solution (*b*). Nitrous oxide is evolved and air drawn through the liquid until all the gas has escaped. The solution is preferably kept in the dark in an ice chest and filtered directly before use.

2. 10 per cent. sulphuric acid solution.

3. N/10 $KMnO_4$ solution.

4. N/10 Sodium oxalate solution.

5. Saturated solution of sodium bicarbonate.

Procedure: A. For Blood: 5 c.c. Trichloracetic acid filtrate are transferred to a 15 c.c. conical centrifuge tube and 2 c.c. of the precipitating reagent added drop by drop to prevent occlusion. The tube is allowed to stand for 30 minutes and then centrifuged for 10 minutes. The supernatant liquid is poured off or treated as in the case of sodium with a micro-syphon. 5 c.c. water are added to the tube without disturbing the precipitate. The tube is centrifuged for 5 minutes and this procedure carried out 3 times. The washed precipitate is dissolved in 10 c.c. warm sodium bicarbonate solution and the solution transferred quantitatively to a 200 c.c. Erlenmeyer flask. 20 c.c. of N/100 $KMnO_4$ solution (prepared from N/10 stock solution when required) are introduced into the Erlenmeyer containing the solution. Dilute sulphuric acid is added till acid, the solution warmed

on the steambath for a few minutes, 20 c.c. freshly prepared N/100 sodium oxalate are introduced and the excess titrated against N/100 $KMnO_4$. Number of c.c. $\frac{N}{100}$ $KMnO_4$ required to oxidise the precipitate, multiplied by 7.1 = Mgm. K per 100 c.c. blood. The constant 7.1 depends on various factors and is readily determined when using a potassium standard as explained by Kramer and Tisdall in the Jnl. of Biol. Chem., Vol. 46, p. 342.

It is not necessary to ash the Trichloracetic acid filtrate, as some workers do, before precipitating the Potassium. This becomes apparent from a consideration of the following:—

TABLE I.

I. (*a*) 5 c.c. T.A.A. filtrate reqd. 7.6 c.c. N/100 $KMnO_4$ solution.

I. (*b*) 5 c.c. T.A.A. filtrate reqd. 7.6 c.c. N/100 $KMnO_4$ solution.

II. (*a*) 5 c.c. T.A.A. filtrate + 1 mg. K. reqd. 21.7 c.c. N/100 $KMnO_4$ solution.

II. (*b*) 5 c.c. T.A.A. filtrate + 1 mg. K. reqd. 21.9 c.c. N/100 $KMnO_4$ solution.

III. 1 mg. K. required 14.1 c.c. N/100 $KMnO_4$ solution.

TABLE II.

A 50 c.c. aliquot of the Trichloracetic acid filtrate of Table I was ashed at a low temperature made up to 50 c.c. with distilled water and the K determined. 5 c.c. solution required 7.7; 7.6; 7.7; 7.7 c.c. N/100 $KMnO_4$ respectively, which compares very favourably with the values obtained in Table I.

This procedure was repeated with another filtrate and before ashing 5 c.c. Trichloracetic acid filtrate (T.A.A.) required 6.2; 6.3; 6.3 c.c. N/100 $KMnO_4$ respectively. After ashing the filtrate and proceeding as before 5 c.c. solution (equivalent to 5 c.c. T.A.A. filtrate) required 6.1; 6.3; 6.3 c.c. $KMnO_4$ solution respectively.

B. for grass: 1 to 2 c.c. of the extract is treated with the precipitating reagent and the procedure for blood followed.

It is not necessary to neutralise the extract before adding the precipitating agent as the following table indicates:—

1 c.c. of grass extract A required without neutralisation 18.2 c.c. N/100 $KMnO_4$ solution.

1 c.c. ,, ,, A required after neutralisation 18.2 c.c. N/100 $KMnO_4$ solution.

1 c.c. ,, ,, B required without neutralisation 16.4 c.c. N/100 $KMnO_4$ solution.

1 c.c. ,, ,, B required after neutralisation 16.5 c.c. N/100 $KMnO_4$ solution.

2 c.c. ,, ,, C required without neutralisation 14.3 c.c. N/100 $KMnO_4$ solution.

2 c.c. ,, ,, C required after neutralisation 14.2 c.c. N/100 $KMnO_4$ solution.

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III.—Determination of Calcium.

Method: Calcium is precipitated as Tricalcium phosphate and the phosphorus determined colorimetrically.

Reagents:

1. 5 per cent. Na_3PO_4 solution—freshly prepared.
2. 18 per cent. NaOH solution.
3. Calcium standard containing .1 mg. Ca per c.c. (Merck's $CaCO_3$ dissolved in dilute HCl and water added to required volume).
4. 1 per cent. Quinol solution, freshly prepared.
5. 20 per cent. sodium sulphite solution.
6. Reagent A. 25 gm. ammoniummolybdate dissolved in 300 c.c. water are added to 75 c.c. concentrated H_2SO_4 previously diluted with 25 c.c. water.
7. Phosphorus standard containing 2 mg. P_2O_5 per 100 c.c. This may readily be prepared (e.g. 200 mgm. per litre) by dissolving .5888 gm. Merck's A.R. microcosmic salt per litre of water. This stock solution containing 200 mgm. P_2O_5 per litre can be diluted to give a standard solution for use corresponding to 2 mg. P_2O_5 per 100 c.c.
8. Washing mixture: 580 c.c. 96 per cent. alcohol and 320 c.c. distilled water are added to 100 c.c. amyl alcohol. This mixture is made distinctly alkaline to phenol-phthalein by adding a few drops of caustic soda solution.

Procedure: A. *for Blood.* 10 c.c. of the Trichloracetic acid filtrate are pipetted into a 20 c.c. centrifuge tube. After adding a drop of phenol-phthalein 18 per cent. caustic soda solution is added drop by drop until alkaline; then 1 c.c. of the caustic soda solution is added. After 5 minutes 1 c.c. of the trisodium phosphate is added and the tube is twirled until the contents are thoroughly mixed. 1.7 c.c. of the calcium standard is treated similarly. The solutions are then allowed to stand for an hour to complete precipitation. The tubes are then centrifuged for ten minutes at 2,500 r.p.m. The supernatant liquid is carefully poured off and the tubes inverted on calcium free filter paper to drain. The mouths of the tubes are wiped with Ca-free filter paper and the precipitates washed by forcibly playing a stream of washing mixture from a wash bottle on them. The precipitate of $Ca_3(PO_4)_2$ is thus broken up when the flow of washing mixture is directed against the sides of the tubes. This operation of washing and centrifuging is done three times.

Finally, the tubes are allowed to drain for 30 minutes when 1.5 c.c. Reagent A is added which should dissolve the precipitate completely. 10 c.c. distilled water, 1.5 c.c. Quinol solution and 1.5 c.c. sodium sulphite are then added successively. As a check upon the calcium standard a phosphorus standard is also prepared by adding to 10 c.c. P. standard (.2 mg. P_2O_5) 1.5 c.c. reagent A, Quinol and sodium sulphite respectively. The calcium standard should read at 29.9 if the phosphorus standard is placed at 30 on the colorimeter scale. Thirty minutes should be allowed for the full development of the colours before colorimetric matching. Calculation: e.g. Ca std. at 30 and unknown reads at 25.

$$100 \text{ c.c. blood contain } \frac{30 \times .17 \times 100}{25 \times 2} \text{ mgm. Ca.}$$

B. *for Grass.* Usually 1 c.c. extract treated as in the case of blood gives colorimetric readings approximating 30 against 1.7 c.c. of a calcium standard at 30 after full development of the colours.

The presence of ferric iron causes no appreciable error in the above determination of calcium in grass extract. This point was tested out by adding four times the quantity of iron usually found in grass to grass extract and determining the calcium in the resulting solution. The biggest error found was 2.5 per cent.

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IV.—Determination of Magnesium.

Method: The calcium present is precipitated as oxalate and subsequently the magnesium as ammonium phosphate after which the phosphorus is determined colorimetrically.

Reagents:

1. The usual reagents for phosphorus, viz. Reagent A, Quinol and sodium sulphite.
2. Magnesium standard; .01 mg. Mg. per c.c. (i.e. .1014 gm. $MgSO_4 \cdot 7\ H_2O$ per litre).
3. 2 per cent. monopotassium phosphate solution.
4. Saturated solution of ammonium oxalate.
5. Strong ammonia Spc. gr. .88 (C.P.).
6. Washing mixture (see under calcium determination).

Procedure: A. *for Blood.* 10 c.c. trichloracetic acid filtrate are transferred to a 20 c.c. centrifuge tube. After the addition of a drop of methyl red, ammonia is added drop by drop until the colour just changes to brown; 1 c.c. of ammonium oxalate solution is then added and the inside of the tube rubbed with a rubber-tipped glass rod until the precipitate of calcium oxalate just begins to form. The rod is rinsed into the tube. A standard containing .07 mgm. Mg is treated likewise and both tubes are set aside for 4 hours at least to complete precipitation.

One c.c. monopotassium phosphate and 2 c.c. of ammonia are added and the tubes again rubbed until the precipitate of Mg NH_4 PO_4 begins to form. After standing for two hours the tubes are centrifuged for 10 minutes at 2,000 r.p.m. The supernatant liquid is poured off and the precipitate washed 3 times and the colours developed as described under the determination of calcium. A phosphorus standard containing .2 mg. P_2O_5 is made as a check and against this at 30 the magnesium standard should read at 29.4 in the colorimeter. The $(COO)_2Ca$ does not interfere with the precipitation of Mg.

$$\text{mgm Mg. in 100 c.c. blood} = \frac{30 \times .07 \times 100}{2 \times \text{average reading}}$$

B. *for Grass*: Generally 1 c.c. of the extract is a suitable aliquot for comparison with 7 c.c. of the Mg. standard. Hence water is added up to 10 c.c. in the tube containing the 1 c.c. extract and the procedure for blood followed.

REFERENCE.

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V.—The Determination of Phosphorus.

Method: The producion of a colour by a phosphate in presence of ammonium molybdate, sulphuric acid and a reducing agent.

Reagents:

1. Reagent A. (Given under Ca determination).
2. Phosphorus standard containing 2 mgm. P_2O_5 per 100 c.c. (given under Ca determination).
3. 1 per cent. freshly prepared Quinol solution.
4. 20 per cent. sodium sulphite solution.

Procedure: A. *for Blood*. The procedure about to be described holds for the inorganic phosphorus fraction of blood only. For total phosphorus, lipoid and total acid soluble phosphorus the method given by Green (1928) is applied. 10 c.c. of the trichloracetic acid filtrate are transferred to a hard glass test tube and 1.5 c.c. of each of the following reagents added successively: reagent A, quinol and sodium sulphite. A standard containing 5 c.c. i.e. .1 mg. P_2O_5 or 10 c.c. i.e. .2 mg. P_2O_5 is prepared simultaneously and the colours compared in a colorimeter after 30 minutes to allow for their full development. A fuller description of this method is given under calcium.

Calculation for 25 mm. reading against .2 mg. std. placed at 30 on the colorimetric scale:

$$\text{100 c.c. blood contains } \frac{30 \times .2 \times .437 \times 100}{25 \times 2} \text{ mgm. P.}$$

It has been found that the procedure just described gives very faint colours for a low value for inorganic phosphorus in the blood, e.g. less than 1 mgm. per 100 c.c. and as blood analysis plays an important rôle in the study of aphosphorosis the method of Kuthner and Cohen, 1927, which gives intense colours even for low values, is followed by some workers here.

Kuthner and Cohen's method for the determination of inorganic phosphorus in blood: —

Reagents:

1. Molybdic sulphuric acid mixture: 1 volume 7.5 per cent. sodium molybdate is mixed with 1 volume 10 N sulphuric acid and two volumes of water. The mixture is stored in a brown, glass stoppered bottle.
2. Stannous chloride stock solution: 10 gm. $SnCl_2$ are dissolved in 25 c.c. concentrated hydrochloric acid and kept in a brown, glass stoppered bottle. .5 c.c. of this solution is diluted to 100 c.c. with distilled water. The diluted reagent does not keep and should be prepared when required. The stock solution lasts about a month.
3. Standard phosphate stock solution: .4394 gm. dried monopotassium phosphate is dissolved in 1 litre distilled water, i.e. 1 c.c. contains .1 mg. P.

A standard phosphate solution is prepared by diluting 5 c.c. of the stock solution to 100 c.c. with distilled water, i.e. .005 mg. phosphorus per c.c. For values between .5 and 1.5 mg. phosphorus per 100 c.c. blood 8 c.c. trichloracetic acid filtrate are pipetted into a hard glass tube. Two c.c. of the molybdic sulphuric acid reagent are added and *while twirling* the tube .5 c.c. diluted $SnCl_2$ solution is introduced. A standard is prepared simultaneously by treating 3c.c. of the diluted standard similarly. Both solutions are made up to 11 c.c. with water and the colours compared in a colorimeter placing the standard at 30 mm. For routine determinations it is best to make up two standards using 3 c.c. of the diluted standard as stated above for comparing solutions of blood which contain approximately 1 mg. P per 100 c.c., and another standard using 6 c.c. of the diluted standard for higher values. For instance, for the fortnightly determination of the inorganic phosphorus of 40 samples of sheep blood here the two standards are made up and 8 c.c. trichloracetic acid filtrate are used. All values up to 4 mg. phosphorus per 100 c.c. blood are then easily read against the nearest of the two standards. For higher values only 4 c.c. trichloracetic acid filtrate are used. It may be added that this method is excellent for comparative work, but is not as accurate as the sulphite-quinol method for individual determinations, unless the unknown is made to read very near the standard on the colorimeter, e.g. 30 against 27 to 33.

Calculation for a reading 25 against .015 mg. standard placed at 30 and using 8 c.c. T.A.A. filtrate.

$$\text{mgm. P. per 100 c.c. blood} = \frac{30}{25} \times \frac{.015}{1} \times \frac{100}{4.6}$$
$$= 1{\cdot}1$$

B. *Total Phosphorus in Grass*: Generally 1 or 2 c.c. of the extract prepared for the determination of P and Cl is a suitable aliquot for comparison with 10 c.c. of the phosphorus standard, i.e. with .2 mg. P_2O_5 by the quinol-sulphite method.

1 or 2 c.c. of the extract is made up to 10 c.c. with water and the procedure for blood followed.

The presence of a little nitric acid in the extract does not interfere with the determination as shown by one of the authors (1931).

REFERENCES.

BRIGGS, A. P. (1924). J.B.C., Vol. 59, pp. 255-259.
GREEN, H. H. (1928). *J. Agric. Sc*,, Vol. 18, pp. 372-375.
VAN DER LINGEN, G. W. B. (1931). This Report.

VI.—The Determination of Chlorine.

Method: Titrimetric determination of chlorine as AgCl.

Reagents:

1. N/10 sodium chloride solution.
2. N/10 silver nitrate solution.
3. N/10 potassium thiocyanate solution.
4. Concentrated nitric acid.
5. Ferric ammonium alum indicator.

Concentrated nitric acid is added to a saturated solution of Ferric ammonium alum until the colour is light brown.

Procedure: A. *for Blood*. 50 c.c. of the trichloracetic acid filtrate are pipetted into a 100 c.c. flask. 5 c.c. concentrated nitric acid and 15 c.c. N/10 $AgNO_3$ are added. The flask is thoroughly shaken but on no account warmed as the trichloracetic acid easily decomposes on heating. The solution is made up to 100 c.c. with water and filtered, using double layers of Whatman No. 40. To 70 c.c. of the filtrate 1 c.c. of ferric indicator is added and N/10 KCNS solution added from a burette drop by drop, gently shaking the flask to prevent occlusion. The end point is sharp.

Calculation: mgm. Cl in 100 c.c. blood

$= 3.545 \quad (15 \times 7/10) - \text{No. of c.c. KCNS used}).$

$\times \frac{100}{7}$

$= 50.64\ (10.5 - \text{No. of c.c. KCNS used}).$

B. *for Grass*: 1 c.c. concentrated nitric acid and 10 c.c. N/10 silver nitrate solution are added to 50 c.c. of the extract prepared for the determination of phosphorus and chlorine in grasses. After warming on the waterbath for a few minutes the solution is filtered through Whatman No. 40 filter paper and the procedure for blood followed.

Summary.

Detailed descriptions are given for the micro-determination of sodium, potassium, calcium, magnesium, phosphorus and chlorine in blood and vegetation.

The authors wish to acknowledge the assistance rendered by all the workers in the Department of Biochemistry in making these methods generally applicable.

Finally, a word of gratitude and appreciation is expressed to Dr. H. H. Green, who began micro-chemical work here, and who is, therefore, largely responsible for its later development.

Studies in Mineral Metabolism XVII.

Phosphorus in the Nutrition of Sheep (2nd Report).

By P. J. DU TOIT, B.A., Dr.Phil., Dr.Med.Vet., Director of Veterinary Services,

A. I. MALAN, D.Sc., Biochemist, Onderstepoort, and

J. W. GROENEWALD, M.Sc.(Agric.), Research Officer, Onderstepoort.

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II. Food consumption.

III. Inorganic phosphorus in the blood.

B. The effect of low Calcium in the ration of sheep on:

I. Food consumption.

II. Increase in weight.

III. Inorganic phosphorus in the blood.

Concluding Remarks.

Summary.

Acknowledgments.

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Appendix.

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INTRODUCTION.

In a preliminary publication in the 16th Report of the Director of Veterinary Services and Animal Industry, Du Toit, Malan and Rossouw gave full details of an experiment which began in August, 1929, with the object of devoting attention to the rôle of phosphorus in the sheep industry. The experiment in question was reported on for the period August, 1929, to January, 1930, and it is now proposed to consider the period January to December, 1930, in this publication.

It will be noticed that a summary of the results is given in charts in preference to tables, which, however, are also appended, for the study of individual records and details of the experiment. For the sake of viewing the experiment in its complete setting and in order to obtain truly comparable charts, the whole period of the experiment has been included; both differences and similarities from the beginning of the experiment show better, while divergencies in the manner in which the groups responded to their respective treatments are more obvious. At the same time it is interesting to see the results already published represented graphically.

It will be remembered that the sheep were divided into groups of ten animals each except group D, where five sheep (D_{11}) were put on a ration low in calcium, the phosphorus content remaining the same as that of group C. As a secondary object of the experiment, however, each group of ten sheep was divided in two sub-groups of five each, and .02 gram potassium iodide was given daily to one of the sub-groups in each of the main groups, except D, which was dealt with as described above. Group A (extreme phosphorus deficiency) then consisted of two sub-groups, A_1 receiving no potassium iodide and A_2 receiving .02 gram KI daily. The same grouping applies to B and C.

At the time of the publication of the preliminary report no differences other than apparently incidental ones could be detected among the strictly comparable sub-groups, for instance A_1 and A_2, one getting KI and the other not, but both being on the same phosphorous ration. It was, therefore, decided to make no division into sub-groups in considering what may be called the broader aspect

of the rôle of phosphorus in the nutrition of sheep. Hence all the sheep in group A_1 and A_2 appeared under the heading Group A, those in B_1 and B_2 under B and those in C_1 and C_2 under C.

However, during the period under consideration in this report, the difference between any two strictly comparable sub-groups—for instance, A_1 and A_2, one receiving KI and the other not, has become so marked that two such sub-groups cannot possibly any longer be regarded as one group of ten. For the purpose of this publication it was decided to leave entirely out of consideration the sub-groups A_2, B_2 and C_2 which received the supplement of KI and to report upon that phase of the experiment at a later date. It means that the groups now consist of five sheep each instead of ten, and that only A_1, B_1, C_1, D_1 and D_2 need be considered.

For the sake of clear presentation and in view of the reduction of the number of sheep in the experiment, the information given on pages 315 to 317 in the preliminary report about the grouping of the sheep, may be summarized as follows:—

I. Group A_1 (5 sheep) receive a ration as low as possible in phosphorus, which amounts to a daily intake of .47 gram.

II. Group B_1 (5 sheep) receive a ration whose phosphorus content is equal to the daily intake of sheep on poor quality pasture which is abundant in phosphorus deficient areas in South Africa. The amount present is 0.73 gram.

III. Group C_1 (5 sheep) receive a ration whose phosphorus content is equal to the daily intake of sheep on excellent quality pasture. This amounts approximately to 1.53 grams.

IV. Group D_1 (5 sheep). The phosphorus content of the ration of those sheep is in excess of their requirements, the daily amount being 2.92 grams.

V. Group D_2 (5 sheep) consume the equivalent of .84 gram calcium in their ration daily, which is a seventh of what these animals would consume on natural pasture of .7 per cent. CaO.

It may be repeated that all the animals were, of course, on the same basal ration and that their intake of minerals except phosphorus and calcium was the same. The figures were calculated on the assumption that sheep of the type used would consume the equivalent of two pounds of dried pasture grass daily when given free access to natural pasture.

Elaborate tables were given in the preliminary report indicating decreased weight, poorer appetite and lower inorganic phosphorus in the blood for the groups receiving a ration deficient in phosphorus. In the present article the results for a further twelve-month period are given and discussed. All conditions with regard to feeding, housing and care of the animals have remained unaltered. Periodic tests for intestinal worms were carried out with negative results and the health of animals inspected daily.

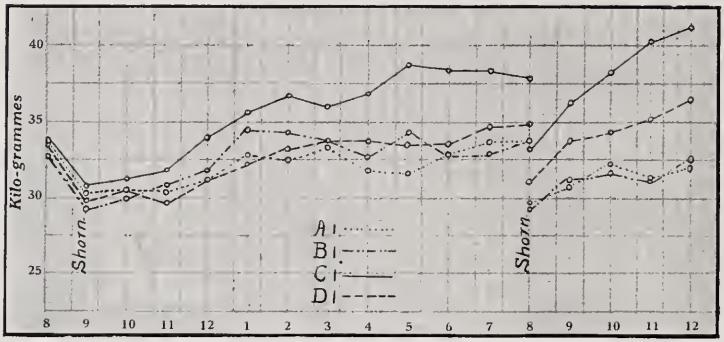
EXPERIMENTAL WORK.

A. The Effect of Varying Quantities of Phosphorus in the Ration of Sheep on:

1. Increase in Weight.

Charts of the average figures are given, illustrating the trend of the various groups with regard to weight, food consumption, and inorganic phosphorus in the blood, while the reader is referred to the appendix for detailed data. The average monthly group weights are given in Figure I.

FIGURE I.



Weights of sheep.

A glance at Figure I indicates that Group C_1 (sufficient phosphorus) made by far the best gains during the course of the experiment. This group responded to a higher phosphorus intake soon after the beginning of the experimental period and continued to show an increased rate of growth throughout. Group C_1 is followed by D_1 (excessive phosphorus), while A_1 and B_1 have done very poorly. The actual average gains or losses made by the different groups covering the whole period are approximately:—

A_1 (extreme P. deficiency) lost 1.5 Kg.
B_1 (P. deficiency) gained 0 Kg.
C_1 (sufficient P.) gained 7.5 Kg.
D_1 (excessive P.) gained 3 Kg.

These groups showed practically no difference in weight at the beginning of the experiment, but at present the average weight per sheep in Group C_1 is 9 Kg. more than that in Group A_1, a remarkable difference indeed when it is remembered that the sheep were almost full grown at the beginning of the experiment.

A glance at the photographs of the groups taken in February, 1931, given below leaves no doubt about the superiority in condition of Group C_1. All the sheep in A_1 are very poor, and it is feared that they may not last until the end of the experiment. B_1 has one sheep in fair condition, but otherwise there is not much to choose between these two groups. It is difficult to say from the photographs where D_1 belongs.

From Figure I it will be seen that it did not take the various groups long to adapt themselves to their experimental conditions. After the first month there appears a period of steady gain in which the Groups C_1 and D_1 gained on the others.

Group A1.—Extreme Phosphorus Deficiency.

Group B1.—Phosphorus Deficiency.

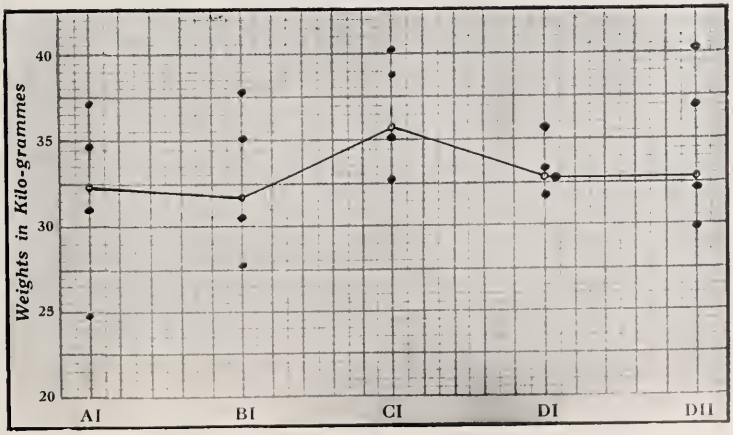
Group C1.—Phosphorus Sufficiency.

Group D1.—Excessive Phosphorus.

Group DII.—Low Calcium.

In order to show how individual sheep in the respective groups responded to their treatment, the monthly weights of each sheep have been averaged for the whole period and given in their respective groups in Figure II. The plotted line joins the medians of the groups.

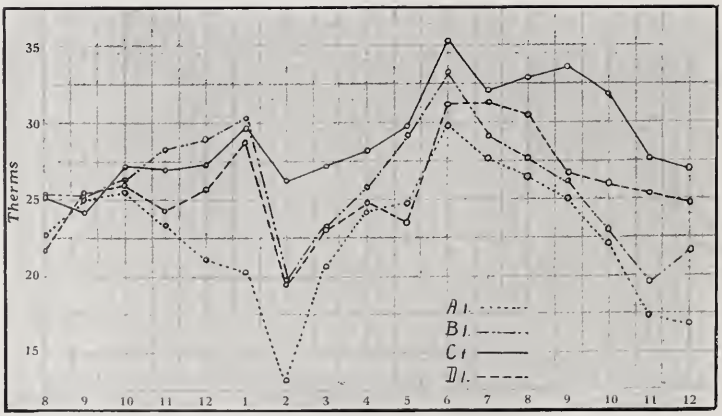
FIGURE II.



When the individual weight averages are considered it will be seen that there is a relatively wide range of weight variation within the different groups. Figure II shows the necessity of working with larger groups of animals, although the variation in some of the groups will probably become less marked as the experiment continues, for there is bound to be a downward trend for all the sheep in groups on phosphorus deficient rations.

Furthermore, another factor must be mentioned in as far as it affects the weight increase of the groups and especially the variations within a group. This factor, viz., reproduction, will be reported on in full detail in the next publication, that is, after two lambing seasons, but as the first lambing period affected the weights in 1930 it will be mentioned here very briefly. The lambs were allowed to remain with their mothers for 21 days and then removed. The extra strain of gestation and lactation upon some of the ewes cannot be ignored. In Group D_1 none of the ewes lambed in 1930, and the variation in individual weights for that group as shown in Figure II is remarkably small. In Group C_1 two lambs were born. The mothers hold the lowest positions in C_1 on the chart in Figure II. In Group B_1 the two ewes showing the poorest average weight in Figure II gave birth to lambs. In Group A_1 no lambs were born. In other words, lambing was no setback to this group. If the effect of parturition and lactation could have been excluded from Groups B_1 and C_1, it seems reasonable to suppose that both Figures I and II would have shown a better weight increase for B_1 and C_1 than they do now. It is of course impossible to gauge the extent of the setback to Group B_1, but the knowledge that two lambs were produced and provided for by two ewes in that group, and none in A_1 leaves very little doubt that the latter group would have struck a poorer figure

FIGURE III.



Food consumption.

than that represented in Figures I and II than Group B_1, if reproduction could have been excluded, or been made to affect A_1 in the same manner as B_1. Similar comments apply to C_1, of course. This matter is important and will be returned to when food consumption is considered, which provides further reason for making the above tentative suggestions.

II. *Food Consumption.*

Charts illustrating the food consumption of the groups appear in Figure III.

It is at once evident from Figure III that there are two main complete fluctuations in the curves. The marked depression in February, 1930, was due to the outbreak of scab (*Psoroptes scabei* var. *oris*) followed by the dipping of the sheep. The increase from February to June may probably be accounted for partly by the recuperation of the sheep after the setback. During May and June the sheep lambed and a gradual decrease set in. Whether the terrific heat during October, November and December, 1930, had anything to do with the decreased appetite or whether it was seasonal, must be left undecided until more data have accumulated. The important fact remains, however, that the sheep in Group C_1 on a diet sufficient in phosphorus have continually consumed more food than those in Groups A_1, B_1 and D_1.

The difference between the food consumption in therms for each group for the first two months of the experiment and the last two months, may be expressed as follows:—

A_1 (extreme P. deficiency) – 6.5 therms

B_1 (P. deficiency) ... ... – 4.5 ,,

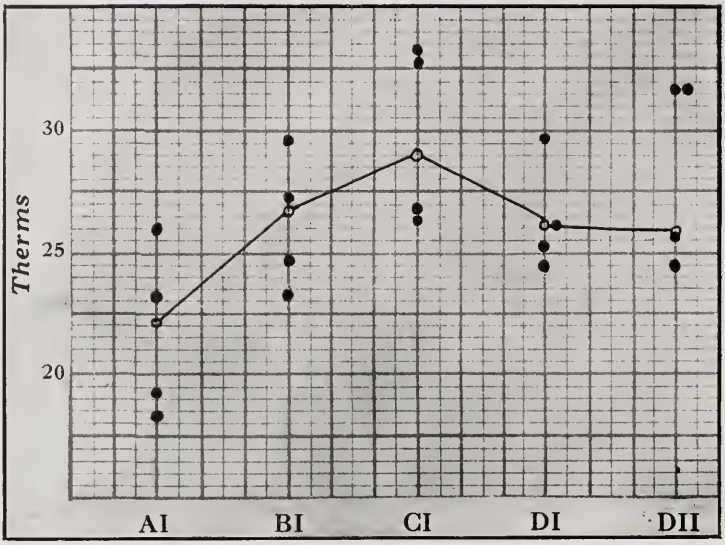
C_1 (P. sufficiency) ... ... + 2.75 ,,

D_1 (Excessive P.) ... ... + 1.5 ,,

The differences are really remarkable, for they bring out one outstanding point beyond doubt, and that is that, although all the animals were given the same amount of food as C_1, those groups on a phosphorus deficient ration consumed appreciably less. It would seem that phosphorus deficiency affects food consumption adversely. This observation with regard to cattle was made by Theiler, Green and du Toit in 1924. In order to show the variations in the food consumption within groups the average amount in therms of food eaten for the total period of the experiment by each sheep is plotted in Figure IV in its respective group.

This curve is very interesting, for it throws further light on some aspects of the weight increases registered by the different groups. If Figures III and IV are studied together there can be no doubt that most food was consumed by the Group C_1 and least by A_1. B_1 showed a slightly better appetite on the whole than D_1.

FIGURE IV.



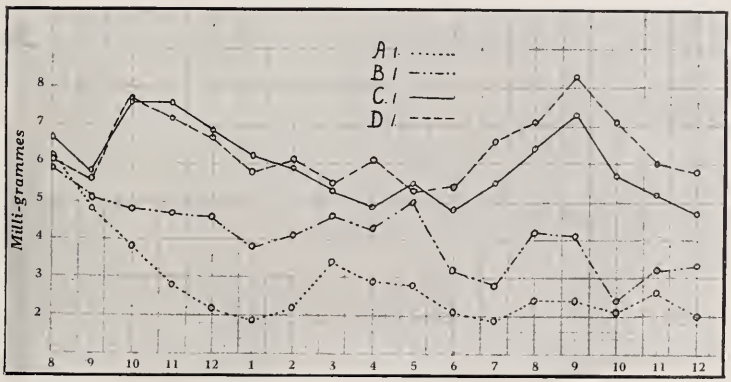
Food consumption.

It is further interesting to compare Figure IV with Figure II. Such a comparison shows quite clearly that, whereas there was no appreciable difference in the average group weights between A_1 and B_1, the food consumption of the latter group was distinctly better than that of the former. This anomaly can be explained in one of two ways: either Group A_1 utilized its food to greater advantage than B_1 or else the latter group was affected by some factor like reproduction which left A_1 unaffected. As already explained, reproduction definitely resulted in a poorer average weight in those groups in which lambing took place. On the other hand, reproduction left food consumption apparently unaffected, for the ewes that lambed in B_1 and C_1 are evenly distributed in those two groups in Figure IV. For instance, the amounts eaten by the two ewes that lambed in Group B_1 are represented by the lowest and the second highest dots in that group. It seems, therefore, that the curve representing food consumption may be accepted more readily as giving the true relative state for all the groups with regard to the question of appetite, while the figures giving the weights of the groups are probably somewhat too high for Groups A_1 and D_1 in relation to B_1 and C_1. This tentative conclusion excludes the possibility of better utilization of food by Group A_1, a matter which cannot be settled until the balance experiments now in progress on the question of the utilization of food as affected by varying quantities of phosphorus in the ration, have been completed.

III. *Inorganic Phosphorus in the Blood.*

A factor of considerable importance was the fortnightly determinations of inorganic phosphorus in the blood of the animals in all the groups. The monthly values plotted in Figure V represent the averages of the two fortnightly determinations for that month.

FIGURE V.



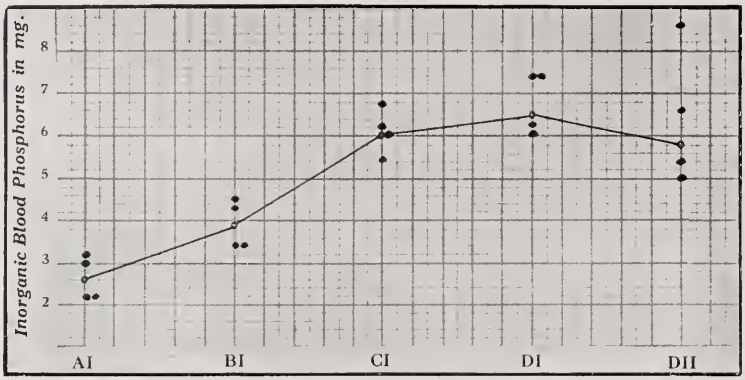
Inorganic phosphorus in mgm. per 100 c.c. blood.

The groups stand out very clearly in Figure V, ranking from highest to lowest in inorganic phosphorus as the ration varies from high to low in its phosphorus content. The curves verify the main statement made in the last publication with regard to this matter, viz. "... that the inorganic phosphorus level of the blood may be taken as being indicative of the degree of phosphorus deficiency of the animal." The greatest drop is seen for Group A_1, of course. The decrease began immediately after the beginning of the experiment, reached the low level of about 2 mgms. per 100 c.c. blood after four months, and remained consistently low throughout. It is strange that all the groups should show a rise in phosphorus in the spring of 1930, whereas during the fall—March to May—both C_1 and D_1 show a depression. Whether these fluctuations are incidental or, may be, seasonal must be left unanswered until a later date. On the whole the inorganic phosphorus in the blood of the sheep of Group D_1 on excessive phosphorus is slightly higher than that of Group C_1, which shows, at all events, a good average figure for blood phosphorus throughout.

The average inorganic phosphorus for individual sheep for the whole experimental period is given in Table VI for the purpose of showing individual variations within the groups.

An extraordinary stability for blood phosphorus, depending on the phosphorus intake, is shown. Animals belonging to the same group show but slight variations, and Figure VI is in all respects an excellent verification of the results plotted in Figure V.

FIGURE VI.

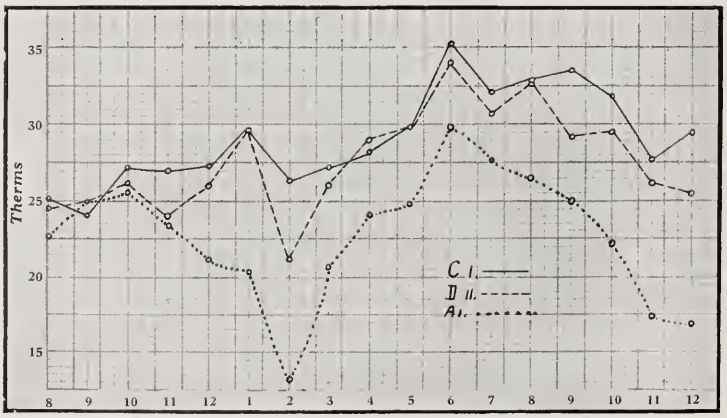


B. The Effect of Low Calcium in the Ration of Sheep on:

1. *Food Consumption.*

It will be remembered from the preliminary report of this work that Group D was divided into two sub-groups D_1, on excessive phosphorus, and D_2 on sufficient phosphorus (as for C_1), but on low calcium. No additional calcium was given and the .84 gram which this group received was that contained in the basal ration.

FIGURE VII.



Food consumption.

Group D_2 is compared in a set of figures with Group C_1 which is on the same diet, but with normal calcium. These two groups have, therefore, only one variant, viz., calcium. A_1 is included in these figures so as to compare the effects of a phosphorus deficiency with those of what seems a calcium deficiency. However, Group A_1 is not strictly comparable with D_2, the difference is that A_1 contains sheep in low phosphorus and normal calcium, whereas the sheep in D_2 are on low calcium and normal phosphorus.

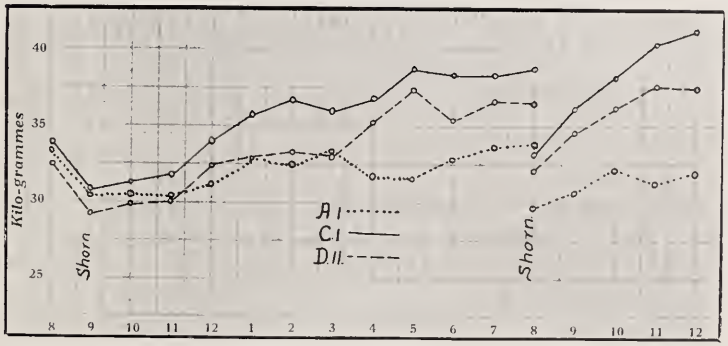
Figure VII gives the food consumption of Groups A_1, C_1, and D_2.

There is only a slight difference in the food consumption of Groups C_1 and D_2, the latter being lower on the whole. Group A_1 showed a very poor appetite when compared with either of the other two groups. It must be added that the food consumption of Group D_2 shown in Figure VII becomes modified somewhat if a glance is taken at Figure IV. D_2 contains two sheep that show a very high consumption of food. The average figure is, therefore, slightly misleading, but can be gauged fairly truly by considering Figure IV as well. The obvious conclusion is that the food consumption of D_2, as a whole, compares less favourably with that of C_1 than Figure VII alone indicates; still there can be no doubt that D_2 showed a better appetite than A_1.

II. *Increase in Weight.*

The average weights of the sheep in Groups A_1, C_1 and D_2, are given in the next figure:

FIGURE VIII.



Weights of sheep.

Weight curves show clearly that the sheep on a low calcium diet in Group D_2 are considerably lighter than those in C_1 which are getting what is considered their normal requirements of calcium. A glance at the photographs of these two groups verifies this statement if general appearance provides at all a way of judging growth.

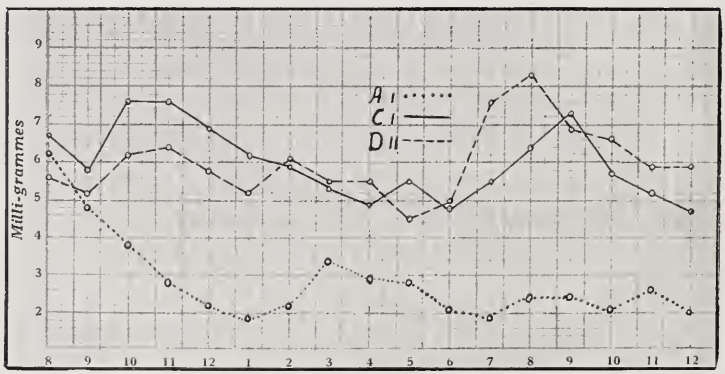
It must be added that three ewes of Group D_2 lambed in June, 1930, which probably means that this group would have shown a more favourable weight increase in comparison with A_1, where no lambs

were born, and even with C_1, where only two lambs were born if reproduction could have been excluded or its effects equalized for all three groups. If Figure II is looked at, and it is borne in mind that reproduction would have given A_1 a poorer position as explained on page 460 it becomes obvious that Group D_2 has done decidedly better than A_1 and approaches C_1 more closely than the figure leads one to believe. In addition, D_2 produced three lambs, whereas in Group C_1 only two were born as already stated. It remains remarkable that sheep receiving as little calcium as those in Group D_2 could have done well at all, and it seems more remarkable that two sheep in that group (see Figure II) could have done almost as well in putting on weight as the two best sheep in Group C_1. The calcium intake of the sheep in Group D_2 was only .84 gram daily, which is approximately equivalent to that of sheep of this type on natural pasture containing .15 per cent. CaO. It must be emphasized that the animals were practically full grown at the beginning of the experiment and that their demand for calcium was probably a minimum. In addition it should be mentioned that these sheep were ferocious earth eaters. Later developments, such as lambing and lactation, will undoubtedly increase the demand for calcium, and it will be interesting to see how the animals will then react. The three ewes that lambed in June, 1930, produced very little milk, insufficient for the needs of the lambs, but retained their conditions remarkably well. This factor will be discussed in the next report dealing with the reproductive side of this experiment in full detail. In the meantime the authors feel that insufficient data are available to explain the remarkable manner in which Group D_2 responded in spite of a diet excessivley low in calcium. A final explanation cannot be attempted until a further period of the experiment has been concluded, which may throw more light on this side of the work.

III. *Inorganic Phosphorus in the Blood.*

The figures for the inorganic phosphorus in the blood Groups A_1, C_1 and D_2 are given in Figure IX.

FIGURE IX.



Inorganic phosphorus in mgm. per 100 c.c. blood.

Groups C_1 and D_2 show no appreciable difference in blood phosphorus, both being high. It is difficult to say whether the low calcium content of the diet affected the absorption and utilization of phosphorus. From Figure IX such a view seems unlikely, but this matter must be left over until the balance experiments already referred to have been completed.

The calcium content of the blood of some sheep in this experiment has been determined. Group C_1, D_1 and D_2 were selected in order to include the greatest variation in the calcium phosphorus ratios of the diets.

In Group A_1 Ca/P = 10.5/1
In Group D_1 Ca/P = 1.65/1
In Group D_2 Ca/P = .55/1

The calcium phosphorus ratio is given here to show the wide range between the different groups; but no importance is provisionally attached to the ratio as such, for, even if detrimental effects had been observed in any of the groups (say A_1 or D_2), such effects should probably have been ascribed, not to the ratio, but to the fact that there was an absolute deficiency of phosphorus in one case (Group A_1) and most probably of calcium in the other (D_2). As a matter of fact, in spite of low calcium, deficient phosphorus or varying calcium phosphorus ratio in the diet of the three groups, the calcium content of their blood remained remarkably constant.

The values for the monthly average of calcium in blood are given in Table I in mgm. per 100 c.c. blood.

TABLE I.

Numbers.	1930.											
	Jan.	Feb.	Mar.	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.
GROUP A_1.—PHOSPHORUS DEFICIENCY. CALCIUM SUFFICIENCY.												
23965.....	—	—	7·8	8·5	8·9	11·5	9·5	8·3	1·0	9·1	—	10·5
24007.....	8·4	9·1	9·0	9·0	8·5	8·8	10·7	9·0	9·3	9·6	—	12·8
23968.....	—	—	7·9	9·3	8·6	9·3	10·4	8·6	9·3	9·5	—	10·8
26153.....	—	—	8·1	8·3	7·8	12·8	9·5	6·9	7·8	9·4	—	8·9
26149.....	8·4	8·8	8·5	8·3	8·5	9·3	10·3	7·8	10·2	9·2	—	10·9
Averages....	8·4	8·9	8·3	8·7	8·5	10·3	10·1	8·1	9·3	9·4	—	10·8
GROUP D_1 P. and CA. SUFFICIENCY.												
23995.....	—	8·0	6·9	7·3	7·3	7·8	8·8	8·2	7·4	8·0	8·1	8·4
23996.....	—	7·1	7·2	7·2	6·6	10·7	7·7	7·2	7·4	7·7	8·4	9·7
23997.....	—	7·1	7·9	7·7	7·3	7·7	8·6	7·7	7·3	7·2	7·4	8·6
23998.....	—	9·0	7·8	8·0	8·3	8·9	9·3	7·7	8·0	8·6	10·1	8·0
26156.....	—	—	7·3	7·8	7·5	10·6	9·7	8·0	8·2	8·4	9·3	9·2
Averages....	—	7·8	7·4	7·6	7·4	9·1	8·8	7·8	7·7	8·0	8·7	8·8
GROUP D_2.—LOW CA. P. SUFFICIENCY.												
23999.....	7·0	7·3	8·0	6·5	5·7	5·9	7·9	6·8	6·8	7·2	12·5	—
24000.....	7·6	8·2	8·0	7·4	7·0	6·3	8·9	8·6	8·7	7·7	9·5	8·9
26158.....	—	—	8·3	7·9	7·5	7·2	—	7·2	7·5	8·6	7·5	9·0
24002.....	7·1	7·3	7·5	6·7	6·9	6·5	7·3	8·1	8·0	7·5	8·0	9·6
24003.....	7·8	6·7	7·1	7·1	9·1	8·3	7·8	6·0	7·3	7·3	8·2	8·8
Averages....	7·4	7·4	7·8	7·1	7·2	6·8	8·0	7·3	7·7	7·7	9·1	9·1

A close study of Table I will indicate that the average figure for calcium in the blood of the sheep in the three groups considered varies somewhat. The highest values are obtained for the sheep in Group A_1 on deficient phosphorus and sufficient calcium, and perhaps the values for Group D_1 on calcium and phosphorus sufficiency are slightly higher than those for the low calcium Group D_2. The differences are in any case not significant enough to warrant serious consideration. Furthermore, it seems unlikely that marked changes can be brought about in the calcium content of the blood as a result of varying the calcium content of the diet. This agrees with the observations of Herzfeld *et al* (1930) in a review article on the value of the calcium content of serum in human beings, that blood calcium remains extraordinarily constant even when disturbances take place in the calcium metabolism of the animal. As a matter of fact the number of cases in which any significant change in the calcium content of the blood was noticed in both disease and health was extraordinarily small. It is certainly doubtful whether the determination of total calcium in blood has any intrinsic value in studies on calcium metabolism. Data is gradually accumulating here from several experiments which, it is hoped, will throw light on this aspect of the problem.

CONCLUDING REMARKS.

Throughout the work it is plain that phosphorus plays a very important part in nutrition. All the charts illustrate the upward trend of a group of sheep supplied with sufficient phosphorus as compared with the downward trend of another group lacking in this element. When phosphorus was supplied in excess (Group D_1) the gain was not as economical as when somewhat less was given (Group C_1). By studying Figures I and VI some idea may be formed as to the probable optimum daily phosphorus requirement for four-tooth Merino ewes under the conditions of the experiment.

It is impossible to form a correct estimate of the gains in all the groups, but certain general conclusions are indicated. Group C_1, on sufficient phosphorus, has undoubtedly done the best so far. Group D_1 on excessive phosphorus is disappointing and Group D_2 on low calcium should perhaps rank next after C_1. A_1 has done very poorly and B_1 is apparently almost on a par with D_1.

SUMMARY.

1. An experiment showing the effects of various amounts of phosphorus in the rations of sheep is reported on for the period January to December, 1930.

2. Sheep getting the phosphorus equivalent of two pounds of good English hay daily show remarkable gains in weight, a good appetite, as well as a normal value for inorganic phosphorus in the blood.

3. Sheep receiving a ration extremely deficient in phosphorus or even one equivalent in phosphorus content to the daily intake of sheep on pasture in the Bechuanaland area have shown no gain in weight, lost appetite, and shown a very low figure for the inorganic phosphorus in the blood.

4. A group of sheep on a low calcium diet have done surprisingly well up to date.

5. Sheep on an excessive amount of phosphorus in the diet have not done as well as sheep on a phosphorus sufficient ration.

6. Inorganic phosphorus in the blood of sheep remains remarkably constant when the phosphorus content of the diet is unchanged. A change within limits of phosphorus in the ration causes almost immediately a change in blood phosphorus.

7. The calcium content of the blood of sheep in three groups shows only slight differences in spite of the fact that the ratio between calcium and phosphorus in the ration of these groups is 10.5/1, 1.65/1 and 0.55/1 respectively.

8. The experiment is being continued and report will be made upon the reproduction and the wool production of the sheep at a later stage.

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Weights of Sheep in Kilograms.

D.O.B. Numbers.	1930.																	
	3.2.	15.2.	1.3.	14.3.	4.4.	15.4.	4.5.	16.5.	2.6.	18.6.	1.7.	15.7.	1.8.	11.8.*	11.8.†	1.9.	7.10.	2.11.	2.12.
							Group	A_1.											
23965	34·1	34·1	34·1	34·1	34·0	33·6	33·6	33·6	34·0	34·1	33·6	34·4	36·7	37·1	32·6	34·1	34·4	34·0	34·1
24007	—	—	37·1	36·7	35·8	36·7	35·8	37·6	37·6	39·0	38·5	39·9	39·4	37·1	34·0	34·1	34·1	34·1	37·1
23968	31·2	30·8	31·2	31·2	31·2	34·1	32·6	32·2	33·6	34·4	34·9	36·2	36·2	34·1	31·2	33·1	33·1	33·6	33·1
26153	—	—	—	27·6	24·0	24·9	23·6	24·5	24·0	25·3	24·0	25·3	24·5	24·0	20·8	20·8	23·1	23·1	23·1
23149	—	—	—	34·0	21·2	32·2	30·3	32·2	33·6	32·2	34·1	34·9	34·4	33·6	30·3	30·8	31·7	32·2	32·6
Average	32·6	32·45	34·1	32·7	31·3	32·3	31·18	32·02	32·56	33·0	33·1	34·14	34·24	33·18	29·7	30·58	33·08	31·4	32·0
							Group	B_1.											
23974	30·3	30·3	30·8	31·2	30·8	31·2	31·2	33·6	31·2	24·0	23·1	24·9	26·7	25·3	22·2	24·0	24·0	24·5	26·3
23975	36·2	35·8	35·8	35·3	35·8	34·9	37·6	32·2	32·2	33·1	34·4	34·4	35·3	34·1	32·2	34·0	35·8	34·9	36·2
23978	36·7	36·7	36·2	35·8	36·7	37·1	39·9	40·8	40·8	41·7	40·8	42·6	42·6	40·8	36·2	37·1	36·2	36·2	38·1
26154	—	—	—	32·6	30·8	32·6	31·7	35·3	31·2	31·2	31·2	33·1	34·4	33·6	28·5	30·3	32·2	31·7	31·7
26152	—	—	—	30·3	28·5	28·5	29·9	32·2	32·2	31·2	31·2	33·1	32·6	32·2	27·6	29·9	29·9	28·5	30·3
Average	34·4	34·26	34·26	33·04	32·5	32·8	34·06	34·8	33·5	32·24	32·24	33·6	34·3	33·2	29·34	31·06	31·6	31·16	32·5
							Group	C_1.											
23985	34·0	34·1	34·1	34·1	36·7	36·2	38·5	41·2	43·0	34·0	34·1	35·3	36·7	35·3	32·2	34·4	36·7	38·5	40·3
23986	37·1	37·6	38·1	37·1	40·3	39·9	43·9	45·3	38·5	38·5	38·5	40·8	42·1	40·8	36·2	40·8	40·8	43·5	43·0
24008	—	—	34·9	34·1	34·1	34·1	34·4	35·3	36·7	37·1	37·1	38·5	38·5	38·5	34·1	36·7	37·1	38·5	39·4
23988	38·5	39·0	39·4	39·4	41·7	43·0	43·0	43·5	45·3	47·1	49·8	42·6	39·4	38·5	33·6	36·7	41·2	42·6	44·8
26159	—	—	—	32·6	31·2	32·2	31·2	31·2	31·2	32·2	33·1	34·0	34·4	34·1	30·3	32·6	35·3	38·1	38·1
Average	36·5	36·9	36·6	35·46	36·8	37·08	38·2	39·3	38·9	37·78	38·5	38·2	38·2	37·4	33·28	36·2	38·2	40·2	41·12
							Group	D_1.											
23995	31·7	32·2	32·6	32·2	28·1	35·3	32·2	32·2	32·2	33·6	33·1	35·3	36·2	35·3	30·8	34·9	37·6	37·6	39·9
23996	33·1	33·6	33·1	33·6	34·4	36·2	33·6	29·9	29·9	31·7	30·3	32·2	33·1	33·6	29·0	29·5	30·8	34·4	37·1
23997	34·4	34·9	34·4	34·4	34·9	36·2	36·2	36·2	38·1	39·0	39·4	40·8	41·7	41·7	36·7	39·9	38·5	34·1	34·1
23998	32·6	3·31	34·0	34·1	36·2	36·2	36·2	36·2	29·9	32·2	32·2	32·2	32·2	32·2	27·6	31·2	30·8	33·1	34·1
26156	—	—	—	31·7	29·9	29·9	30·8	31·2	34·1	34·4	36·2	35·8	37·1	36·2	31·2	33·1	34·1	36·7	37·1
Average	32·9	33·45	33·5	33·2	32·7	34·76	33·8	33·14	32·8	34·18	34·2	35·26	36·06	35·8	31·06	33·7	34·36	35·18	36·46
							Group	D_2.											
23999	36·7	36·7	36·2	36·2	38·5	41·7	43·0	43·5	35·3	47·5	48·0	45·3	44·8	45·3	40·8	43·5	45·3	47·5	45·3
24000	34·1	34·4	34·1	34·1	35·8	38·5	38·5	40·3	42·1	43·9	38·5	39·4	40·8	39·9	36·2	38·5	40·8	41·2	42·6
24002	32·2	32·6	33·6	32·6	36·2	37·6	39·4	39·0	32·2	31·7	34·1	34·4	34·9	32·2	28·5	31·2	32·2	34·1	32·6
24003	29·5	29·5	29·5	29·5	31·2	33·1	33·1	34·1	29·0	26·3	28·1	30·3	29·9	29·9	26·7	28·5	30·8	33·1	34·0
26158	—	—	—	30·8	29·0	30·3	30·8	32·2	32·6	33·1	33·6	34·9	34·4	33·1	28·5	31·2	32·6	32·2	33·1
Average	33·1	33·3	33·35	32·6	34·14	36·2	36·9	37·8	34·2	36·5	36·46	36·8	37·0	36·08	32·14	34·58	36·3	37·6	37·5

* Before shearing. † After shearing.

Food Consumption. Rations in Kilograms. Energy in Therms.

D.O.B. Numbers.	8/1/30 to 8/2/30.		9/2/30 to 8/3/30.		9/3/30 to 7/4/30.		8/4/30 to 7/5/30.		8/5/30 to 7/6/30.		8/6/30 to 9/7/30.		10/7/30 to 9/8/30.		10/8/30 to 10/9/30.		10/9/30 to 8/10/30.		9/10/30 to 8/11/30.		9/11/30 to 10/12/30.	
	Hay.	Fanko.	Hay.	Fanko.	Hay.	Fanko.	Hay.	Fanko.	Hay.	Fanko.	Hay.	Fanko.	Hay.	Fanko.	Hay.	Fanko.	Hay.	Fanko.	Hay.	Fanko.	Hay.	Fanko.
											GROUP	A_1.									
26153.....	3·71	7·1	2·28	4·49	4·25	6·76	4·91	10·27	3·56	8·58	3·84	10·06	4·05	10·27	4·94	9·31	4·86	8·48	4·89	7·71	5·73	6·25
23965.....	4·67	12·46	3·94	10·70	4·76	11·41	3·64	10·39	3·15	8·47	5·75	11·72	5·56	14·57	5·31	13·77	4·62	11·87	6·04	10·01	4·85	6·65
26149.....	1·92	5·84	1·32	2·70	4·66	8·39	5·00	10·80	4·92	13·94	5·57	14·54	5·12	12·68	5·53	13·51	5·80	12·85	5·24	8·91	5·53	6·88
24007.....	2·71	9·55	1·76	3·56	4·82	8·92	3·03	11·08	3·99	13·11	4·57	14·58	5·54	10·52	6·49	8·91	5·14	11·28	5·66	11·33	6·64	8·77
23968.....	3·21	9·81	3·77	7·32	3·94	8·56	3·16	10·72	3·00	10·77	4·38	14·88	4·67	12·34	5·36	11·47	4·93	9·55	4·84	8·64	5·06	7·55
Averages.....	3·24	8·96	2·61	5·73	4·48	8·80	3·95	10·65	3·72	10·98	4·82	13·16	4·99	12·08	5·52	11·39	5·07	10·81	5·33	9·32	5·56	7·22
Therms.....	1·60	18·70	1·30	11·90	2·20	18·40	1·93	22·27	18·2	22·90	2·35	27·50	2·44	25·20	2·71	23·80	2·52	22·5	2·60	19·5	2·72	15·09
Therms.....	20·3		13·2		20·6		24·2		24·72		29·85		27·64		26·51		25·02		22·1		17·81	
											GROUP	B_1.									
23974.....	6·62	12·65	3·51	10·46	6·71	10·72	7·27	7·26	6·72	8·47	5·46	10·60	6·87	11·44	8·12	9·48	7·07	7·37	7·07	7·38	7·37	6·98
23975.....	4·98	12·64	1·12	6·89	4·04	11·18	3·89	10·78	4·58	14·47	5·23	17·10	4·68	15·63	5·93	14·97	5·00	11·21	5·16	9·45	4·99	8·08
23978.....	7·72	14·10	3·76	10·03	6·65	12·22	6·73	13·97	9·02	17·14	8·77	16·59	7·31	11·41	6·28	11·98	6·27	10·82	6·44	11·96	5·13	9·91
26154.....	7·00	12·87	3·92	10·72	5·74	9·11	6·02	11·79	5·53	10·27	5·81	13·47	6·86	10·67	7·53	10·12	6·82	13·02	6·37	10·36	6·88	8·20
26152.....	6·91	12·49	1·95	7·17	6·01	5·24	5·00	11·45	5·81	12·36	5·22	14·42	6·13	13·06	6·60	11·96	6·13	12·91	5·37	9·06	4·93	6·93
Averages.....	6·64	12·95	2·85	8·83	5·83	9·69	5·78	11·05	6·33	12·54	6·10	14·44	6·37	12·44	6·89	11·70	6·26	11·07	6·08	9·58	5·86	8·02
Therms.....	3·00	27·10	1·4	18·5	2·8	20·3	2·82	23·01	3·08	26·2	2·98	30·18	3·11	26·01	3·15	24·46	3·06	23·14	2·95	20·04	2·85	16·77
Therms.....	30·3		19·9		23·1		25·83		29·28		33·16		29·12		27·62		26·2		22·99		19·62	
											GROUP	C_1.									
26159.....	8·40	11·84	5·82	11·20	6·33	8·42	5·43	11·36	6·31	7·85	7·04	13·21	7·57	11·44	8·94	11·61	7·91	14·66	7·46	13·14	7·33	11·11
23985.....	7·20	11·67	3·32	8·72	6·63	11·48	6·51	12·16	7·58	13·60	7·06	13·87	8·37	15·40	7·20	14·60	7·79	15·33	7·30	14·37	7·58	10·94
23986.....	6·96	14·04	3·34	11·49	6·27	14·41	4·95	15·12	6·38	15·98	7·77	17·58	7·25	16·13	8·59	17·10	8·21	14·93	7·08	14·30	8·45	12·81
24008.....	7·63	9·78	5·00	12·19	4·64	8·23	4·44	8·36	6·06	11·79	6·74	14·18	6·17	12·61	7·23	13·62	6·54	11·37	5·89	12·13	7·05	9·85
23988.....	8·05	14·90	4·59	14·01	7·18	15·60	7·09	13·70	6·63	14·47	7·44	17·31	6·66	13·02	8·33	14·88	7·48	15·05	7·40	14·45	8·73	12·10
Averages.....	7·64	12·45	4·41	11·52	6·21	11·62	5·68	12·14	6·71	12·74	7·21	15·23	7·20	13·72	8·05	14·36	7·58	14·27	7·02	13·68	7·82	11·36
Therms.....	3·7	26·02	2·2	24·1	3·03	24·3	2·76	25·3	3·25	26·6	3·53	31·8	3·52	28·6	2·97	30·02	3·71	29·83	3·43	28·5	3·83	23·75
Therms.....	29·7		26·3		27·33		28·06		29·85		35·33		32·12		32·99		33·54		31·93		27·58	
											GROUP	D_1.									
23995.....	4·64	11·46	2·27	10·94	5·74	11·54	5·88	8·34	5·72	9·39	6·98	12·10	8·0	14·86	8·85	14·94	7·19	15·09	7·23	14·81	8·19	12·47
23996.....	8·60	13·90	3·73	8·88	6·44	12·24	6·86	9·69	6·04	3·60	6·21	9·85	8·37	10·81	8·88	8·75	6·99	10·61	7·14	12·37	8·67	10·68
23997.....	6·70	13·86	2·20	7·22	6·37	10·09	5·73	12·01	6·40	11·80	7·78	17·00	8·18	15·32	7·73	16·98	6·32	9·15	4·17	2·82	7·48	5·46
23998.....	7·69	10·80	4·32	9·11	7·18	11·48	7·04	12·03	6·91	10·80	8·24	11·62	8·56	8·34	8·36	9·33	7·71	9·29	7·82	11·07	9·20	10·74
26156.....	6·56	11·06	4·49	7·44	6·49	2·28	7·33	9·80	7·21	13·17	7·74	15·29	8·06	15·79	8·77	12·88	7·45	11·46	7·72	13·22	8·21	11·45
Averages.....	6·83	12·22	3·40	8·54	6·44	9·52	6·57	10·37	6·45	9·75	7·39	13·17	8·23	13·03	8·51	12·58	7·13	11·12	6·81	10·86	8·35	10·16
Therms.....	3·3	25·5	1·7	17·8	3·1	19·9	3·21	21·6	3·14	20·39	3·61	27·53	4·02	27·23	4·16	26·29	3·48	23·25	3·33	22·7	4·08	21·2
Therms.....	28·8		19·5		23·0		24·81		23·53		31·14		31·25		30·45		26·73		26·03		25·32	
											GROUP	D_2.									
23999.....	5·89	13·90	1·92	11·82	5·39	14·54	5·47	15·20	6·46	14·88	7·15	17·07	8·22	17·33	8·98	17·11	7·34	15·58	6·76	15·11	7·24	7·88
24000.....	7·81	13·18	3·08	10·88	5·34	13·31	4·97	13·19	6·40	15·84	6·73	16·42	6·33	15·28	6·80	16·83	6·33	14·95	6·47	14·60	7·09	11·21
24002.....	5·89	12·27	3·45	9·74	6·45	10·98	6·15	12·52	4·93	11·78	6·59	14·31	6·71	10·18	7·47	12·59	6·52	10·52	6·75	10·01	6·69	6·83
24003.....	5·93	11·98	3·02	7·63	6·93	9·70	7·09	10·20	5·66	9·93	4·80	12·93	6·32	11·00	8·39	11·30	6·71	11·37	6·44	14·15	8·08	10·39
26158.....	7·70	11·72	4·31	7·06	7·68	6·44	6·92	10·92	6·05	11·79	6·72	13·45	7·16	11·32	6·37	11·77	4·78	10·40	6·28	9·02	6·73	12·14
Averages.....	6·64	12·61	3·15	9·43	6·36	10·99	6·12	12·40	5·90	12·84	6·40	14·80	6·95	13·03	7·60	13·92	6·33	12·56	6·54	12·58	7·16	9·69
Therms.....	3·2	26·4	1·5	19·7	3·1	22·9	2·95	25·93	22·88	26·85	3·12	30·93	3·39	27·23	3·7	29·1	3·05	26·26	3·1	26·27	3·49	20·26
Therms.....	29·6		21·2		26·0		28·88		29·73		43·05		30·62		32·82		29·31		29·48		23·75	

Inorganic Phosphorus in Milligrams per 100 c.c. Blood.

1930.

D.O.B. Numbers.	27.1.	10.2.	17.3.	31.3.	14.4.	28.4.	9.5.	26.5.	7.6.	23.6.	7.7.	28.7.	11.8.	25.8.	8.9.	22.9.	12.10.	27.10.	10.11.	24.11.	8.12.
Group A_1.																					
23965	1·5	2·1	2·8	2·6	2·0	2·5	1·8	2·1	2·2	1·9	1·4	1·4	1·6	1·8	3·3	1·6	2·1	1·5	1·7	2·7	1·8
24007	—	—	3·0	3·5	2·5	2·2	—	2·5	2·0	2·0	1·9	2·5	2·4	2·5	2·7	2·3	2·5	1·5	1·7	3·3	1·7
23968	2·0	2·2	2·7	2·6	2·2	2·1	2·1	2·3	2·0	1·4	2·0	1·6	2·6	1·7	2·2	1·8	2·1	1·5	2·0	3·1	2·2
26153	—	—	5·6	2·3	3·2	4·3	4·3	3·0	2·5	2·2	2·3	2·2	3·1	2·0	2·2	2·3	2·8	2·4	2·7	3·3	2·0
26149	—	—	5·6	3·9	4·0	4·7	3·7	3·1	—	2·5	2·1	2·0	2·8	3·4	1·6	2·4	2·7	2·1	2·2	3·2	2·5
Average	1·75	2·15	3·9	2·98	2·78	3·16	2·97	2·6	2·17	2·0	1·94	1·94	2·5	2·28	2·4	2·08	2·44	1·8	2·06	3·12	2·04
Group B_1.																					
23974	3·9	4·2	3·9	2·8	1·9	2·8	5·3	4·1	2·0	0·9	1·7	3·0	4·2	3·4	2·9	2·6	2·4	2·3	2·8	3·2	3·6
23975	3·1	3·8	3·9	5·0	2·9	4·4	5·6	3·1	2·7	2·8	1·9	2·6	3·1	2·8	2·9	2·6	1·9	2·1	2·6	3·0	2·6
23978	4·4	4·3	2·8	5·5	5·6	6·6	6·8	4·5	5·2	4·2	4·0	2·7	3·8	4·4	3·6	4·1	3·6	2·3	3·2	4·0	2·8
26154	—	—	4·5	5·9	4·9	5·3	5·7	3·9	—	4·1	2·1	3·5	5·5	5·7	7·3	4·7	2·0	2·2	2·8	4·3	3·6
26152	—	—	5·9	6·4	4·3	4·9	6·9	4·2	3·6	3·3	3·8	3·1	3·4	5·5	5·8	4·1	2·0	2·8	3·2	2·9	4·0
Average	3·8	4·1	4·2	5·12	3·9	4·8	6·06	3·96	3·37	3·06	2·7	2·98	4·0	4·36	4·5	3·6	2·38	2·34	2·9	3·48	3·32
Group C_1.																					
23985	6·2	5·7	4·1	5·8	2·8	6·0	5·0	5·2	5·2	3·1	6·1	4·6	6·9	8·9	8·7	6·9	5·0	6·0	6·4	4·6	5·8
23986	6·0	4·8	4·0	4·1	2·5	4·7	6·0	3·8	4·0	4·0	5·2	5·8	8·2	6·3	6·6	6·3	6·7	5·7	6·6	8·0	4·8
24008	—	—	5·4	6·7	4·2	5·5	7·2	4·9	5·0	5·7	5·3	5·3	5·4	6·0	7·5	5·2	5·1	6·6	5·0	4·2	4·5
23988	6·2	7·2	4·6	5·8	5·9	5·5	6·0	4·9	4·9	3·7	4·2	3·6	3·1	5·6	8·2	8·2	6·6	7·5	5·1	5·3	5·2
26159	—	—	6·2	6·3	6·7	6·1	6·6	5·0	7·3	5·0	6·1	8·5	7·1	6·3	8·7	6·7	4·2	4·0	4·2	2·8	3·3
Average	6·3	5·9	4·8	5·7	4·4	5·56	6·16	4·76	5·28	4·3	5·38	5·56	6·14	6·6	7·94	6·66	5·52	5·96	5·46	4·98	4·72
Group D_1.																					
23995	5·2	5·5	5·9	5·8	6·5	7·5	7·8	5·0	6·2	4·8	5·6	6·2	8·5	6·3	8·7	7·3	6·8	6·9	6·9	5·8	5·7
23996	4·7	6·7	4·0	5·1	7·4	5·3	7·4	4·3	5·5	4·1	8·5	6·8	8·0	6·6	8·7	7·3	5·6	7·3	5·6	6·1	5·6
23997	6·6	5·5	5·1	5·5	4·6	7·9	5·1	5·1	7·2	5·2	7·5	6·2	7·3	6·7	9·4	7·1	8·0	9·4	5·0	5·7	6·0
23998	6·4	6·7	6·1	5·3	4·6	7·1	5·2	4·3	6·8	6·0	7·3	7·7	9·0	5·7	9·4	10·5	7·2	8·7	6·6	6·6	7·2
26156	—	—	5·8	6·5	5·7	4·2	5·1	3·5	4·7	3·6	4·9	4·9	6·6	5·8	8·2	6·0	5·7	5·3	6·9	5·0	4·6
Average	5·7	6·1	5·38	5·64	5·76	6·4	6·1	4·44	6·08	4·74	6·76	6·36	7·88	6·22	8·88	7·62	6·66	7·52	6·2	5·84	5·82
Group D_2.																					
23999	6·7	6·7	6·0	7·6	6·3	7·4	7·3	5·0	7·7	6·7	7·4	5·8	10·9	12·5	8·3	6·0	9·4	9·4	5·2	6·8	6·9
24000	4·6	5·4	5·0	4·4	4·5	4·2	3·7	3·7	5·2	4·5	4·8	5·2	5·5	7·3	8·7	5·0	5·4	5·7	6·6	5·5	4·8
24002	4·7	5·5	4·7	5·9	4·5	4·9	5·2	2·6	4·4	3·9	10·6	9·5	10·1	4·8	6·6	6·6	6·6	6·2	6·0	6·6	8·6
24003	4·9	6·6	5·0	5·4	6·0	5·9	4·8	2·6	4·5	3·9	6·5	12·1	11·9	8·3	10·0	7·1	7·9	7·3	3·8	6·6	5·1
26158	—	—	6·8	4·0	—	5·7	6·3	4·3	4·9	4·6	—	6·5	6·5	4·9	6·6	4·7	4·2	3·9	5·2	6·5	4·2
Average	5·2	6·05	5·5	5·46	5·3	5·62	5·44	3·64	5·34	4·72	7·3	7·82	8·98	7·56	8·04	5·88	6·7	6·5	5·36	6·4	5·92

The Preparation of Cystine.

By J. G. LOUW, M.Sc., Chemist, Department of Biochemistry, Onderstepoort.

Considerable importance is at present attached to the effect of the sulphur-containing amino acid, cystine, on the growth of wool or hair.

Since nutritional experiments on the problem are in progress at Onderstepoort, it became necessary to prepare cystine on a fairly large scale, as the commercial product was too expensive for general use. Furthermore, it was found that the preparation of cystine as described in most text-books was not quite as straightforward as the authors contended. Apart from this, wool was abundant and large quantities of "hydrolysate" were required in another portion of the experiment. It was found after some practice that cystine could be prepared at the rate of about 20 grams per day while, in addition, abundant hydrolysate is obtained for the requirements of the experiment.

The method about to be described is a modification of Folin's method and cystine can be prepared at a cost of approximately one-third of that of the commercial product and quite free from tyrosine, whereas the purchased sample was found to be contaminated with this amino acid.

Two and a half kilograms of scoured wool are boiled in a fifteen-litre pyrex flask, fitted with a reflux condenser, for 15 hours with four litres of concentrated hydrochloric acid and one litre of water.

As all the wool cannot be introduced at the start, portions of 100 grams are forced into the acid, the mixture gently heated over wire gauze and the wool allowed to dissolve before further additions.

At the end of the period of boiling the hydrolysate is allowed to cool and 3.5 litres of 10 *N* caustic soda solution are slowly added with continual shaking. On cooling, cystine, salt and some tyrosine separate out. The acidity is still well below pH 3.6, but no more cystine will separate out on further addition of caustic soda solution.

The precipitate is filtered off on a large Buchner funnel, taken up with enough water to dissolve all the salt and again filtered. The combined filtrates still contain about 0.6 per cent. cystine, as determined by Folin and Marenzi's method, and are used for other purposes as stated.

The precipitate is then dissolved in 1.5 litres hot 20 per cent. (by vol.) hydrochloric acid, the solution allowed to cool and filtered. Owing to the presence of a considerable amount of wool grease, the filtering is a very slow process and is best allowed to proceed overnight using ordinary funnels.

The filtrate, a dark solution, is adjusted to pH 3.6 with concentrated ammonia. Care should be taken not to neutralise beyond this point, as the precipitated cystine will then be contaminated with tyrosine which begins to separate out round about pH 3.6.

The mass, containing crude cystine, is filtered through a large Buchner funnel and re-dissolved in 1 litre 20 per cent. HCl. This solution is heated to boiling with 50 grams of Merck's medicinal charcoal and filtered. If a clear solution is not obtained, heating with another 30 grams of charcoal will usually give a perfectly clear solution. The addition of concentrated ammonia to this solution until a pH just below 3.6 is reached precipitates the cystine as an amorphous white product.

To obtain the characteristic hexagonal crystals of cystine, the amorphous powder can be dissolved in 1 litre 20 per cent. HCl and diluted to 2 litres with water. The cystine may then be precipitated with ammonia as before.

The cystine, i.e. the amorphous powder or the hexagonal crystals, is filtered off, repeatedly washed with small quantities of distilled water followed by 96 per cent. alcohol and finally with ether, dried at 100° C. in an oven and weighed. A yield of about 120 grams cystine is usually obtained.

Summary.

A method is described for the preparation of pure cystine for experiments on the effect of cystine in the diet on the growth of wool and hair.

REFERENCES.

FOLIN, O. (1910). On the Preparation of Cystine. *J.B.C.* 8, 9-10.

FOLIN, O., AND MARENZI, A. D. (1929). An Improved Colorimetric Method for the Determination of Cystine in Proteins. *J.B.C.* 83, 103-108.

The Effect of Small Quantities of Nitric Acid on the Colorimetric Determination of Phosphorus.

By G. W. B. VAN DER LINGEN, M.Sc., Department of Biochemistry, Onderstepoort.

Nitric acid is often present in plant extracts whose phosphorus content has to be determined. Theoretically fair quantities of this acid will interfere with the reduction of the ammonium-phosphomolybdate by quinol and sodium sulphite which will result in faulty determinations. It was, therefore, deemed advisable to find out whether extracts containing small quantities of dilute nitric acid could be used for the colorimetric determination of phosphorus. This was undertaken in view of the determination of inorganic elements in a large number of grass samples. Two extracts of each sample would suffice for all the analyses, provided that phosphorus and chlorine could be done on the same extract, which incidentally contained nitric acid. The nitric acid used in making the grass extract was approximately 7.79 N. Hence this strength of acid was taken for the determinations given in Table I. A phosphorus standard containing 2 mg. P_2O_5 per 100 c.c. was used. Concentrated nitric acid diluted with an equal volume of water gave the required strength.

Table I.

Number.	c.c. P.stand.	c.c. 7.79 N HNO_3.	c.c. H_2O.	Av. Col. Reading.	Per Cent. Error.
Standard.....	10	0	2	Set at 30	—
1.....	10	·25	1·75	30·3	— 1·0
2.....	10	·50	1·50	31·4	— 4·7
3.....	10	·75	1·25	32·3	— 7·9
4.....	10	1·00	1·00	33·5	— 11·7
5.....	10	1·50	·50	36·3	— 21·0
6.....	10	1·75	·25	40·6	— 35·3
7.....	10	2·00	·00	48·5	— 61·7

The determinations were carried out according to the method described by Green (1928).

From the accompanying graph it appears that the presence of less than .25 c.c. of 7.79 N HNO_3 introduces a negligible error in the determination.

To test out this point more carefully .779 N HNO_3 was used and the experiment repeated.

TABLE II.

Number.	c.c. P.stand.	c.c. ·779 N HNO_3.	c.c H_2O.	Average Reading.	Per Cent. Error.
Standard.....	10	0	2	30	—
1.....	10	·5	1·5	30	0
2.....	10	1·0	1·0	30	0
3.....	10	1·5	·5	30·1	— ·3
4.....	10	2·0	0	30·1	— ·3

It must be emphasized, however, that the values given under "% Error", probably differ from the "true percentage error" by about .5 to 1 per cent., since the average fluctuations in the readings amounted to between .2 and .3 mm. at a colorimeter setting of 30 mm.

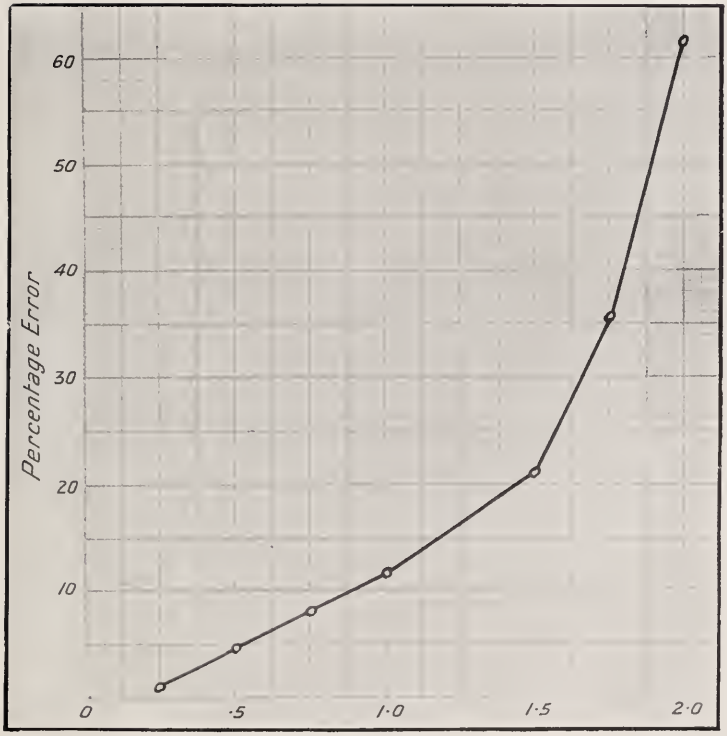
These results indicate the adaptibility of the nitric acid extract, used in the estimation of the chlorine content of grasses, to the estimation of the phosphorus content, provided not more than .25 c.c. of 7.79 N HNO_3 is present in the solution prior to the development of the colours.

SUMMARY.

Small quantities of dilute nitric acid (less than 2.0 c.c. of .779 N HNO_3) do not interfere with the colorimetric determination of phosphorus when quinol and sodium sulphite are used as reducing agents.

REFERENCES.

GREEN, H. H. (1928). Studies in Mineral Metabolism, IV. Determination of Phosphorus Compounds in Blood by Dry Combustion. *Jnl. Agric. Sc.*, Vol. 18, pp. 372-375.



c.c. 7.79 N. HNO_3.

The Increase of Inorganic Phosphorus in Blood Containing Lithium-Citrate as Anticoagulant.

By G. W. B. VAN DER LINGEN, M.Sc., Department of Biochemistry, Onderstepoort.

In connection with field work it was felt that if blood samples collected in bottles containing lithium citrate as anticoagulant showed no, or but a slight increase of inorganic phosphorus within, say, four or five days, blood for analysis could easily be forwarded in this manner to Onderstepoort.

Although we are concerned with whole blood, it is interesting to note that Kay and Robinson (1924) found an increase of 20 per cent. in the inorganic phosphorus of the plasma of unlaked blood kept at a temperature of 37° C. for 5 hours. Robinson and Martland (1924) again only found a slight increase in the inorganic phosphorus of unlaked blood kept at room temperature for 6 hours.

The following experiments were undertaken:—

A. Five samples of blood were drawn from cows in bottles containing 1 c.c. of 20 per cent. lithium citrate solution to each 100 c.c. of blood. The proteins were precipitated half an hour after the blood was drawn, using 5 per cent. trichloracetic acid and the inorganic phosphorus determined by the method described by Green (1928). After four hours the inorganic phosphorus was again determined. As is indicated in the following table the hydrolysis is quite appreciable.

Room Temperature 24° C.

	½ hour.	4 hours.	Percentage Increase.
No. 1	6·18	6·64	7·4
No. 2	6·68	7·10	5·9
No. 3	7·08	7·49	5·8
No. 4	7·45	7·77	4·3
No. 5	8·08	8·53	6·6

Since the blood clotted after standing for about 24 hours, the same type of experiment was repeated, but instead of 1 c.c., 2 c.c. of lithium citrate were added per 100 c.c. blood.

AVERAGE ROOM TEMPERATURE 24.5° C.

No.	½ hour.	4½ hours.	Percentage Increase in 4½ hours.	24 hours.	29 hours.	53 hours.	77 hours.	96 hours.	144 hours.
1.....	8·0	8·0	0	8·04	7·99	9·56	9·70	*	*
2.....	7·71	8·19	6·2	8·55	8·55	10·16	10·16	10·92	*
3.....	6·80	7·37	8·5	7·44	7·58	8·92	8·92	*	*
4.....	6·30	6·54	3·8	7·08	7·08	8·29	8·52	9·72	11·9
5.....	6·46	6·90	6·8	6·90	8·84	8·96	11·24	12·10	14·52

* Signifies that the blood clotted.

The accompanying graph indicates to what extent the inorganic phosphorus increased during a period of six days.

It is obvious that, apart from the danger of clotting taking place during transport, dependable results for inorganic phosphorus cannot be obtained if blood is kept even for comparatively short periods after bleeding.

SUMMARY.

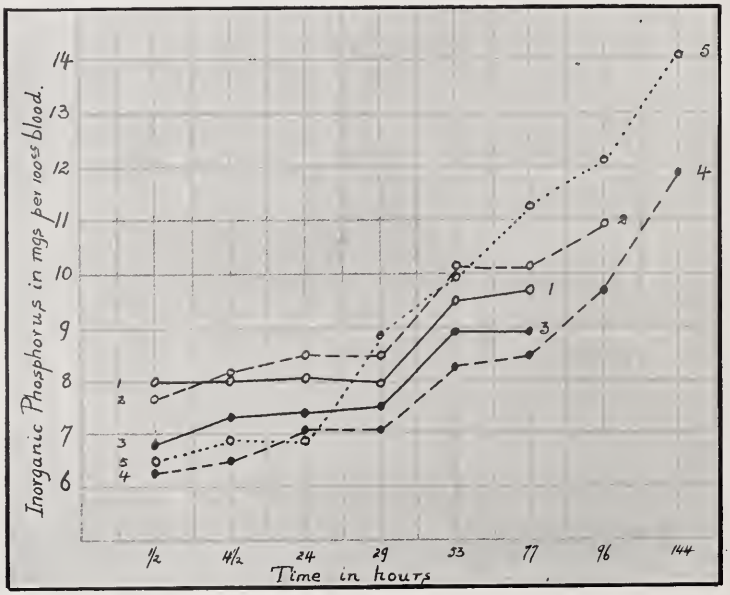
When lithium citrate is used as anticoagulant the increase in inorganic phosphorus in blood is quite appreciable even after 24 hours.

REFERENCES.

GREEN, H. H. (1928). *Jnl. Agric. Sc.*, Vol. 18, pp. 372-375.

KAY, H. D., AND ROBISON, R. (1924). *Biochem. Jnl.*, Vol. 18, pp. 755-764.

ROBISON, R., AND MARTLAND, M. (1924). *Biochem. Jnl.*, Vol. 18, pp. 765-768.



The Effects of Sulphur on Merino Sheep.

By D. G. STEYN, B.Sc., Dr.Med.Vet., Veterinary Research Officer, Onderstepoort.

As a result of experiments with antidotes in connection with sulphur and bietou (Dimorphotheca) poisoning,* sulphur is now being recommended as an efficient preventive for the prussic acid form of "geilsiekte". Hence it was thought necessary to determine the dose of sulphur which could be administered with safety over prolonged periods to sheep. Ordinary flowers of sulphur was used in this investigation.

Preliminary Experiments.

Sheep were dosed as follows:—

Group I.—Each of five sheep received 7.5 grams of sulphur three times weekly (Tuesday, Thursday, and Saturday) for a period of four months.

Group II.—Each of five sheep received 15 grams of sulphur three times weekly (as above) until symptoms of poisoning appeared.

Two sheep were kept as controls. The controls and the animals in Group I developed no symptoms of ill-health. The five sheep in Group II developed symptoms of sulphur poisoning on the thirty-fifth day of the experiment.

The *symptoms* exhibited by these animals were: Inappetence, cessation of rumination, extreme apathy, diarrhoea, weakness, hurried and superficial respiration, an accelerated and weak pulse and paralysis.

In spite of the fact that the dosing was immediately discontinued, four of the five affected animals died within three to ten days after the appearance of symptoms.

The post-mortem appearances were as follows:—

Sheep 20998.—Stomach and intestines on being opened emitted a strong smell of hydrogen sulphide; slight fatty changes in liver and kidneys.

* *15th Annual Report, Director of Veterinary Services*, October, 1929, p. 783.

Sheep 21195.—Slight hyperaemia of lungs; fatty changes in the kidneys; stomach and intestines on being opened emitted a strong smell of hydrogen sulphide.

Sheep 21013.—Marked oedema and hyperaemia of lungs; pronounced subendocardial haemorrhages; fatty changes in the kidneys; haemonchosis and oesophagostomiasis.

Sheep 20964.—Stomachs and intestines on being opened emitted a strong smell of hydrogen sulphide; chronic croupous duodenitis and jejunitis with sulphur particles firmly adhering to the inflamed mucosa.

Unchanged sulphur particles were found mixed with the gastro-intestinal contents of the above sheep.

Histology.

Sheep 20964.—The liver showed slight fatty changes and the kidneys hyperaemia and slight fatty infiltrative changes in the epithelium of the cortex.

Conclusions.

From the foregoing experiment, it may be concluded that 22.5 grams of sulphur per week per sheep administered in daily doses of 7.5 grams over a period of four months, have no deleterious effect on the health of the animals, whereas 45 grams per week per sheep over a period of thirty-five days given in daily doses of 15 grams cause symptoms of poisoning and high mortality.

Subsequently, the undermentioned experiment was conducted in order to determine the safe dose of sulphur for sheep, and also to note whether the prolonged dosing of sulphur in the quantities given below would have harmful effects on the wool en weight of the experimental animals:—

Group A.—Each of five sheep received 5 grams of sulphur once weekly (Monday) for a period of twelve months.

Group B.—Each of five sheep received 5 grams of sulphur three times weekly (Monday, Wednesday, and Friday) for a period of twelve months.

Group C.—Each of five sheep received 5 grams of sulphur six times weekly (every day except Sundays) for a period of twelve months.

Group D.—Five controls not receiving sulphur ran under the same conditions as Groups A, B, and C.

TABLE I.—GROUP A.

Each sheep received 1.25 draehms (5 grams) of sulphur every Monday for the period 13th November, 1929, to 11th November, 1930.

No. Age.	14186 Full Mouth.		15305 Full Mouth.		24668 Full Mouth.		24673 Full Mouth.		24550 Full Mouth.	
Sex.	Ewe.		Ewe.		Wether.		Wether.		Wether.	
Date of Weighing.	Weight in lb.	Increase.	Weight in lb.	Increase.	Weight in lb.	Increase.	Weight in lb.	Increase.	Weight in lb.	Increase.
1929.										
13.11	95·5	—	84	—	80·5	—	85	—	86	—
27·11	99	3·5	85	1	86	5·5	85·5	·5	90	4
11.12	102	6·5	86·5	2·5	87	6·5	90	5	94	8
25.12	106·5	11·5	89	5	87·5	7	91·5	6·5	94	8
1930.										
8.1	106·5	11·5	89	5	90	9·5	93·5	8·5	94	8
22.1	113·5	18	93	9	93	13·0	94·5	9·5	96·5	10·5
5·2	113·5	18	96·5	12·5	95	14·5	95	10	100	14
19·2	118	22·5	99	15	99	18·5	100	15	100	14
5.3	118	22·5	100	16	99	18·5	100	15	103	17
19.3	123	27·5	102	18	100	19·5	102	17	105	19
3.4	125	29·5	103·5	19·5	102	21·5	103	18	107·5	21·5
17.4	125·5	30	108·5	24·5	104·5	24	105	20	107·5	21·5
30.4	126·5	31·5	106	22	105·5	25	106	21	108·5	22·5
14.5	129	33·5	107	23	104·5	24	106	21	108	22
28.5	—	—	—	—	—	—	—	—	—	—
11.6	129·5	34	110	26	108	27·5	106	21	105	19
25.6	129	33·5	112	28	106	25·5	103·5	18·5	100	14
9.7	129	33·5	115·5	31·5	108	27·5	107	22	99	13
23.7	127·5	32	112	28	108	27·5	106·5	21·5	90·5	4·5
6.8	128	32·5	116	32	110	29·5	105	20	83	3
20.8	126·5	31	115·5	31·5	110	29·5	104·5	19·5	75·5	10·5
4.9	125·5	30	120	36	110	29·5	105	20	This animal died on	
19.9	130	34·5	121	37	114·5	34	107·5	22·5	30.9.30 and will be	
3.10	126·5	31	118	34	114	33·5	108·5	23·5	referred to in the	
17.10	128·5	33	120	36	116	35·5	111	26	course of this article.	
Date of shearing	11.11.30		11.11.30		11.11.30		11.11.30		11.11.30	
Weight of 1-year-old clip	11·5		9·5		10·5		9·5		—	
Average weight of clip					10·25					

All weights are given in pounds.

TABLE II.—GROUP B.

Each sheep received 1.25 drachms (5 grams) of sulphur every Monday, Wednesday, and Friday for the period 13th November, 1929, to 11th November, 1930.

No. / Age.	24221 Full Mouth.		9128 Full Mouth.		18558 Full Mouth.		24519 Full Mouth.		24379 Full Mouth.	
Sex.	Ewe.		Ewe.		Wether.		Wether.		Wether.	
Date of Weighing.	Weight in lb.	Increase.	Weight in lb.	Increase.	Weight in lb.	Increase.	Weight in lb.	Increase.	Weight in lb.	Increase.
1929.										
13.11	69	—	97·5	—	65	—	93	—	88·5	—
27.11	71·5	2·5	100	2·5	69·5	4·5	96·5	3·5	90·5	2
11.12	74·5	5·5	101·5	4	73	8	99·5	6·5	94	5·2
25.12	79·5	10·5	102	4·5	77·5	12·5	97	4	96·5	8
1930.										
8.1	78	9	103	5·5	81	16	100	7	98·5	10
22.1	81	12	105·5	8·5	83	18	102	9	102	13·5
5.2	84	15	107·5	10	89·5	24·5	107·5	14·5	105	16·5
19.2	85·5	16·5	105·5	8	89·5	24·5	111	18	104·5	16
5.3	87·5	18·5	107·5	10	92	27	115	22	106·5	18
19.3	87	18	109	11·5	96	30	115	22	108	19·5
3.4	86	17	109·5	12	97	32	117	24	109·5	21·
17.4	93	24	109	11·5	97·5	12·5	117	24	108·5	20
30.4	92·5	23·5	111·5	14	96·5	31·5	118	25	112	23
14·5	98·5	29·5	112	14·5	99	34	120	27	115·2	27
28.5	—	—	—	—	—	—	—	—	—	—
11.6	99·5	30·5	115	17·5	102·5	37·5	123·5	30·5	116	27·5
25.6	99	30	115·5	18	105	40	125	32	118	29·5
9.7	101	32	114·5	17	107	42	129	36	120·5	32
23.7	99·5	30·5	112	14·5	107·5	42·5	128·5	35·5	122	33·5
6.8	100	31	116	18·5	108	43	128·5	35·5	123·5	35
20.8	97·5	28·5	115	17·5	108	43	127·5	34·5	123·5	35
4.9	102	33	116	18·5	110·5	45·5	131	38	126·5	38
19·9	103·5	34·5	118·5	21	110	45	134	41	132·5	40
3.10	104	35	117	19·5	115	50	133·5	40·5	128·5	40
17.10	106	37	120.5	23	121.5	56.5	136.5	43·5	This animal died on 10.10.30 and will be referred to in the course of this article.	
Date of shearing	11.11.30		11.11.30		11.11.30		11.11.30		11.11.30	
Weight of 1-year-old clip	13		12		11		15·5		—	
Average weight of clip					12·9					

All weights are given in pounds.

TABLE III.—GROUP C.

Each sheep received 1.25 *drachms* (5 *grams*) *of sulphur every day, except Sundays, for the period* 13*th November*, 1929, *to* 11*th November*, 1930.

No. / Age.	21642 Full Mouth.		23406 Full Mouth.		10047 Full Mouth.		23683 Full Mouth.		24420 Full Mouth.	
Sex.	Ewe.		Ewe.		Wether.		Wether.		Wether.	
Date of Weighing.	Weight in lb.	Increase.	Weight in lb.	Increase.	Weight in lb.	Increase.	Weight in lb.	Increase.	Weight in lb.	Increase.
1929.										
13.11	66	—	88·5	—	112	—	71	—	81·5	—
27.11	72·5	6·5	94	5·5	113·5	1·5	80	9	86·5	5
11·12	78	12	101·5	13	114·5	2·5	84	13	90·5	9
25.12	81	15	104	15·5	117	5	87	16	94	12·5
1930.										
8.1	83·5	17·5	106	17·5	115·5	3·5	89	18	99·5	18
22.1	89	23	110	21·5	121·5	9·5	88·5	17·5	98	17·5
5.2	89·5	23·5	115	26·5	121·5	9·5	99	28	102·5	21
19.2	92·5	26·5	117	28·5	125·5	13·5	100·5	29·5	103·5	22
5.3	96	30	120	31·5	127	15	102·5	31·5	104	22·5
19.3	99·5	33·5	123·5	35	131·5	19·5	106	35	107	25·5
3.4	100	34	124.5	36	132	20	107·5	36·5	107·5	26
17.4	104	38	125.5	37	130	18	108	37	108	26·5
30.4	106·5	40·5	125	36·5	130·5	18.5	107.5	36·5	109	28·5
14.5	107	41	125	36·5	130·5	18·5	109	38	113·5	32
28.5	—	—	—	—	—	—	—	—	—	—
11.6	112·5	46·5	128·5	40	135	23	110·5	39·5	113·5	32·5
25.6	112.5	46·5	130	41·5	135	23	110	39	117	35·5
9·7	117	51	132·5	44	134·5	22·5	118	47	115	33·5
23.7	116	50	This animal was		133	21	120	49	115.5	34
6.8	116·5	50·5	killed on 15.7.30		136	24	124	53	113·5	32
20.8	121	55	and will be referred		137.5	25.5	125.5	54·5	111·5	30
14.9	122	56	to in the course of		138·0	26	126·5	55·5	113	31·5
19.9	124	58	this article.		136·5	24·5	126	55	110·5	29
3.10	123	57			139·5	27·5	125	54	111	29·5
17.10	126·5	60·5			137	25	125	54	111·5	30
Date of shearing	11.11.30		—		11.11.30		11.11.30		11.11.30	
Weight of 1-year-old clip	12·5		—		9		16		11·5	
Average weight of clip					12·25					

All weights are given in pounds.

TABLE IV.—GROUP D.

Controls—running under same conditions as Groups A, B, and C, but receiving no sulphur.

No.	17876		7647		22986		24733		24418	
Age.	Full Mouth.		Full Mouth.		Full Mouth.		Full Mouth.		Full Mouth.	
Sex.	Ewe.		Ewe.		Ewe.		Wether.		Wether.	
Date of Weighing.	Weight in lb.	Increase.	Weight in lb.	Increase.	Weight in lb.	Increase.	Weight in lb.	Increase.	Weight in lb.	Increase.
1929.										
13.11	116	—	100·5	—	55	—	90·5	—	74	—
27.11	116	0	100·5	0	58·5	3·5	97	6·5	79	5
11·12	116	0	102·5	2	64	9	100	9·5	78	4
25.12	116.5	·5	103	2·5	63·5	8·5	100·5	10	82	8
1930.										
8.1	116	0	104·5	4	65	10	102·5	12	84	10
22.1	117	1	105	4·5	65·5	10·5	103·5	13	85·5	11·5
5.2	122·5	6·5	104·5	4	61	6	106	15·5	89	15
19.2	121	5	110	9·5	66	11	113	22·5	87·5	13·5
5.3	124·5	8·5	110	9·5	69	14	113·5	23	91·5	17·5
19.3	128	12	111	10·5	69·5	14·5	117	26·5	92	18
3.4	129	13	112·5	12	72	17	117·5	27	94	20
17.4	127·5	11·5	112·5	12	73	18	121	30·5	93	19
30.4	130·5	14·5	112·5	12	70	15	122·5	32	94	20
14.5	131	15	115·5	15	74	19	126.5	36	95	21
28.5	—	—	—	—	—	—	—	—	—	—
11.6	132	16	116·5	16	80	25	125·5	35	97·5	23·5
25.6	133·5	17·5	116·5	16	78	23	127·5	38	99	25
9.7	136	20	117·5	17	77·5	22·5	129·5	39	100	26
23.7	134·5	18·5	115·5	15	80	25	127·5	37	99·5	25·5
6.8	135	19	117	16·5	85	30	129·5	39	99·5	25·5
20.8	135·5	19·5	116·5	16	85·5	30·5	128·5	38	102	28
4.9	135·5	19·5	115·5	15	91	36	130·5	40	105·5	31·5
19.9	133·5	17·5	115	14·5	87·5	32·5	126·5	36	105	31
3.10	134	18	118·8	18·5	90	35	125·5	35	104	30
17.10	129·5	13·5	120	19·5	95·5	40·5	125·5	35	106·5	32·5
Date of shearing	11.11.30		11.11.30		11.11.30		11.11.30		11.11.30	
Weight of 1-year-old clip	10		7		8·5		10		11	
Average weight of clip					9·3					

All weights are given in pounds.

TABLE V.

(Extracted from Tables I, II, III, and IV.)

COMPARATIVE TABLE.

D.O.B. No.	Original Weight of Sheep After Shearing.		Increase in Weight during First 3 Months of Experiment.	Average Increase in Weight during First 3 Months of Experiment.	Increase in Weight during First 6 Months of Experiment.	Average Increase in Weight during First 6 Months of Experiment.	Increase in Weight during First 9 Months of Experiment.	Average Increase in Weight during First 9 Months of Experiment.	Increase in Weight during First 12 Months of Experiment.	Average increase in Weight during First 12 Months of Experiment.	Average Wool Clip for Each Group.
14186	95·5	Group A, Table I.	18		31		31		33		
15305	84		12·5		22		28		36		
24668	80·5		14·5	14	25	24·3	27·5	27	35·5	32·6	10·25 lb.
24673	85		11		21		21·5		26		
24550	86		14		22·5		—		—		
24221	69	Group B, Table II.	15		23·5		30·5		37		
9128	97·5		10		14		14·5		23		
18558	65		24·5	16	31·5	23	42·5	31·3	56·5	40	12·9 lb.
24519	93		14·5		25		35·5		43·5		
24379	88·5		16·5		23·5		33·5		40		
21643	66	Group C, Table III.	23·5		40·5		50		60·5		
23406	88·5		26·5		36·5		—		—		
10047	112		9·5	21·7	18·5	31·9	21	38·5	25	42·4	12·25 lb.
23683	71		28		36·5		49		54		
24420	81·5		21		27·5		34		30		
17876	116	Controls, Group D, Table IV.	6·5		14·5		18·5		13·5		
7647	100·5		4		12		15		19·5		
22986	55		6	9·4	15	18·7	25	24·2	40·5	28·2	9·3 lb.
24733	90·5		15·5		32		37		35		
24418	74		15		20		25·5		32		

FIG. 1.—Sheep 21642, which has received 30 grams of sulphur weekly for 12 months.

FIG. 2.—× Sheep 17876 (control) and Sheep 21642.

FIG. 3.—The group of sulphur-dosed sheep.

From Table V, which is compiled from the results given in Tables I, II, III, and IV, it will be seen that the average increase in weights of Groups A, B, and C during the first three months of the experiment was 14 lb., 16 lb., and 21.7 lb. respectively, and 9.4 lb. in Group D (Controls). The increase in weight during the first six months was 24.75 lb., 23 lb., 31.9 lb., and 18.7 lb. respectively, and during the first nine months 27 lb., 31.3 lb., 38.5 lb., and 24.2 lb. respectively. The increase in weight during the first twelve months was 32.6 lb., 40 lb., 42.4 lb., and 28.2 lb. for the Groups A, B, C, and D respectively.

It will be noticed that Sheep 22986, 24733, and 24418 of the control group were 4-tooth animals, whereas the rest of the animals in the experiment were full-grown. These three sheep weighed originally 55, 90.5, and 74 lb. respectively. Their respective increases in weight for the period of twelve months were 40.5, 35, and 32.5 lb., whereas those of the remaining two full-grown sheep (17876 originally weighing 116 lb. and 7647 originally weighing 100.5 lb.) were 13.5 and 19.5 lb. respectively.

The fact that three immature sheep were included in the control group renders the increases in weight of the sheep which received sulphur even more striking when compared with the control group.

The accompanying graph presents the results graphically and perhaps more strikingly.

Furthermore, it will be noted that the increase in weight in Groups A, B, and C was, relatively speaking, much greater during the first three months than during the last nine months of the year.

In connection with the clip it will be seen that the sheep which received the sulphur (Groups A, B, and C) yielded an average of 10.25 lb., 12.9 lb., and 12.25 lb. respectively, whereas the controls yielded an average of 9.3 lb.

Sheep No. 24550 (Group A) during the process of dosing inhaled sulphur on the 187th day of the experiment. The animal coughed badly and within a month after the inhalation it commenced losing in weight. At death the animal weighed 10.5 lb. less than when it was placed in the experiment, in spite of the fact that it had gained 22 lb. during the first six and a half months. It died ten and a half months after the commencement of the experiment.

Both the macroscopic and microscopic lesions were typical of "jaagsiekte".

Sheep 24379 (Group B) was found dead in the pen eleven months after the commencement of the experiment. No symptoms of disease had been noted in this animal.

Post-mortem Appearances.—Obesity; marked fatty changes in the liver, heart, and kidneys; numerous subcapsular haemorrhages in kidneys. Oedema and chronic venous congestion of the lungs; erosion and ulceration of the bladder; one urinary calculus (6 mm. in diameter) in the bladder, and one in the urethra just cranial to the sigmoid flexure causing complete occlusion with retention of urine.

Sheep 23406 (Group C) had to be killed eight months after commencement of the experiment on account of calculi causing complete obstruction of the urethra.

Discussion.

From the above experiment it appears that sheep can take sulphur at the rate of five grams six times weekly over prolonged periods with strikingly beneficial results to their weight and wool yield. Sulphur when administered in these quantities appears to act as an alterative and constructive.

In regard to the quality of the wool, it was found that there was a marked improvement both in that of the sheep which received sulphur and that of the controls.

There was no difference in the cystine-content of the wool samples collected prior to and after dosing with sulphur for twelve months.

Sheep 24550 (Group A) died from "jaagsiekte" four months after it had inhaled sulphur. Is this a coincidence or did the sulphur play a part in the production of "jaagsiekte" in this case?

Sheep 24379 (Group B) and 2340 (Group C) developed urinary calculi. Dosing with sulphur is still being continued, and it is hoped to elucidate the part played by sulphur in the production of these calculi.

On the day of shearing it was noticed that all the wethers suffered from a purulent inflammation of the prepuce.[1] Within three weeks after shearing all the animals had recovered spontaneously.

(1) Compare my article on "Pisgoed", *16th Report of D.V.S. and A.I.*, Aug., 1930, p. 417.

It may be added here that some farmers hold that sheep which have access to sulphur licks or are dosed with sulphur develop skin diseases when exposed to wet weather. The sheep employed in Groups A, B, C, and D were exposed to several heavy showers and to drizzly weather in the course of this experiment, and up to the present none of them have developed skin lesions.

The sheep employed in the foregoing experiments were chosen at random, and before any definite conclusions can be drawn about the effects of sulphur on the quantity and quality of the wool and on the weight of the sheep, it is advisable to conduct experiments on the lines given below.

All the animals should be obtained from the same locality, or preferably should be born at the locality where the experiments are to be conducted. This is essential in order to exclude the possibility of obtaining sheep from areas differing in the quality of the grazing. Animals obtained from areas poor in grazing will, when put on good daily diet, gain more weight than animals accustomed to good grazing, and favourable changes in this respect might erroneously be attributed to the feeding or dosing of sulphur. Animals of the same sex, age, and type should be employed, and in studying the effects of sulphur on the wool, sheep with the same class of wool should be employed.

Furthermore, it would be of interest to study the effects of sulphur on sheep at different ages and on pregnant and lactating ewes.

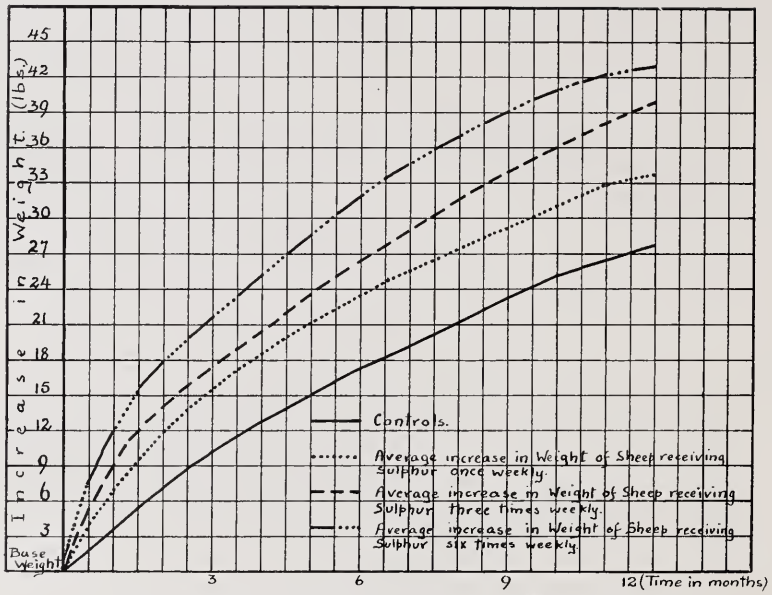
Conclusions.

Sulphur can be administered to Merino sheep at the rate of five grams once, three times, or six times weekly, over prolonged periods, with striking beneficial effects on their weight and wool yield. Furthermore, it has been established that the above amounts of sulphur given throughout the course of a year, have no detrimental effect on the quality of the wool and do not cause an increase in the cystine content of the wool.

Acknowledgments.

I am indebted to Mr. Schuurman, Chief of the Sheep and Wool Division, Union Buildings, for dividing the sheep into the different groups, and to Mr. Louw, M.Sc., of the Department of Bio-chemistry, Onderstepoort, for determining the cystine content of the wool samples.

I also wish to place on record my appreciation of the pains taken by Mr. L. Roux, Sheep and Wool Expert, Nooitgedacht Experimental Farm, Ermelo, in examining the wool samples collected at the beginning and at the end of the experiment.



Graph Illustrating the Effects of Sulphur on Merino Sheep.

Section VI.

Pathology.

P. J. J. Fourie ...	The Haematology and Pathology of Haemonchosis in Sheep.
G. de Kock	Studies on the Blood of Mice.
G. de Kock, P. J. du Toit and D. G. Steyn	Studies on the Aetiology of Dunsiekte or Enzootic Liver Disease of Equines in South Africa.
J. Quin	Photosensitising Influence of Haematoporphyrin on Sheep and Goats.

17th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1931.

The Haematology and Pathology of Haemonchosis in Sheep.

By P. J. J. FOURIE, M.R.C.V.S., Sub-Director of Veterinary Services, Onderstepoort.

Thesis approved by the University of Utrecht, Holland, for the Doctorate in Veterinary Science.

INTRODUCTION.

In the literature available, no reference to a complete and systematic study of the haematology of sheep infected with wireworm (*Haemonchus contortus*) has been found. That anaemia develops as a result of the action of the gastro-intestinal worms, is well known; and, under field conditions, pale mucous membranes and the most extreme degrees of badly staining and watery blood, are features which are commonly associated with the effects of internal parasites. How the anaemia is brought about, and what part individual species of parasites play in its production, are points that have not been properly elucidated.

The main object of this study is the pathogenesis of the anaemia. In such an investigation it is necessary to produce in worm-free sheep, under properly controlled experimental conditions, a progressive and preferably fatal anaemia, by the administration of pure faeces cultures of the parasites. The subject matter of this work can conveniently be sub-divided into:

(1) General Survey and Plan of Work.

(2) Technique.

(3) Details of Experiments:

- (i) Haematology and Pathology of Haemonchosis in Sheep.
  - A. Fatal cases of Haemonchosis.
  - B. Recovery cases and those in which no effects were observed.
  - C. Controls.
- (ii) Pathogenesis of the Anaemia in Haemonchosis of Sheep.

(4) Some unusual lesions of Haemonchosis.

(5) Summary.

GENERAL SURVEY AND PLAN OF WORK.

Weekly observations on the blood were recorded, before infection and throughout the experiment, until the animal died as a result of the parasitic infestation. In order that reliable conclusions can be drawn as to the effects produced in animals by particular parasites, it is necessary (1) that the animal host at the time of the commencement of the experiment be free from infestation, and (2) at the time of the completion of the experiment no other parasites than the one under investigation be recovered at post-mortem examination. These are the ideal conditions that must be aimed at, but it must be confessed that only rarely are they completely attained in practice, notwithstanding that the most elaborate precautions are strictly maintained.

Veglia (1928) describes in detail the method employed by him for rearing and maintaining worm-free lambs at this Institute. He removes the lambs from their mothers soon after birth, hand-rearing them in specially constructed cages with raised floors. In this way it is possible to control more or less successfully the worm infection of particular animals, but it was impossible to prevent this entirely, although such infestation with parasites other than those with which the experimental animals were infected was present only to a small and negligible extent.

Sixteen lambs reared in this way by Veglia were used in the first series of these experiments. Later on, when no more such lambs were available, young sheep, entirely free from worm infestation, were bought from certain farms in the Karroo. The complete absence of worm infestation was determined by making post-mortem examinations of some animals that were born and reared under the same conditions as others used for these experiments and was confirmed before these animals were actually drafted into an experiment by careful faeces cultures. This work was courteously directed by my colleagues, Drs. Veglia and Mönnig.

Lambs or young sheep were selected for these experiments because of the generally accepted view that young animals are more susceptible than are older animals to the effects of parasites. Hadwen (1925), referring chiefly to Ascariasis in horses, maintains that this is due to an immunity or tolerance acquired or developed by older animals as a result of infection with parasites. Young animals, not having had an opportunity to develop this power of tolerance, suffer severely when they become infected. This immunity and tolerance is due to anti-substances and eosinophiles, both of which function in neutralizing the cast-off products of the worms. He believes that the eosinophiles secrete, in addition to these agents which neutralize effete products of worms, a substance which paralyses or kills the worms themselves. He states that the percentage of eosinophiles in the circulation may be low, or even altogether absent in severe cases of parasitism. There is an apparent leucopenia as far as the eosinophiles are concerned, but this is due to their withdrawal from the circulation and their concentration around the parasites in the tissues. Similarly, an eosinophilia may mean that the parasites have been overcome. In such cases the excess of eosonophiles has again returned to the circulation (from which they gradually disappear).

The use of young lambs for these experiments has the additional advantage that they are less likely to be seriously infested with any parasite other than the one under investigation.

It is naturally essential that pure cultures of the parasites should be used for infecting experimental animals. One may have the good fortune to obtain a pure culture from a sheep that happens to harbour the pure infestation of the parasite that it is intended to use. In cases of mixed infestation, undesirable parasites can be destroyed by specific drugs, which must not be parasiticidal for the parasites which are to be cultivated or a pure culture can be obtained by keeping a sample of mixed faeces culture until the unwanted larvae have died out. Mönnig (1930) shows that eggs of *Trichostrongylus* spp. containing complete embryos are more resistant to desiccation than such eggs of *Haemonchus contortus*, *Oesophagostomum columbianum* and *Strongyloides papillosus*. In the same paper this author discusses a successful method of producing a pure infestation of Oesophagostomes in sheep by administering the adult nodular worms in saline per rectum.

A further method consists in infecting worm-free sheep with adult males and females of the parasite. In some cases eggs are passed in the faeces in due course, and in this way a pure culture can be obtained with which further clean animals can be infected, and rich cultures from these can be used for gross infection of experimental animals.

Recently Stoll (1929) described an ideal method for the natural infection of animals on the pasture. Natural infection of the pasture is introduced by means of lambs which have been given larvae developed from eggs obtained from a single female wireworm. If it is possible to prevent gross infestation of the pasture by other parasites, one should with this method be able to study the haematology of haemonchosis under natural conditions. This is very desirable, as none of the animals artificially infected with wireworm and kept under controlled experimental conditions in the stable developed any oedematous swellings, e.g. "bottle jaw," which are characteristic clinical features of verminosis.

The experimental animals were infected by injecting a saline suspension of larvae, obtained from faeces cultures, into the mouth by means of a hypodermic syringe without the needle.

Pure faeces cultures of the parasites having been obtained on the one hand and clean animals to infect on the other, it was thought that no great difficulty would be experienced in producing typical cases for haematological study. Accordingly a fairly ambitious programme was drawn up for the comparative study of the blood of a number of animals infected at the same time with pure cultures of parasites commonly encountered in the gastro-intestinal tract of sheep under natural conditions in South Africa. These are (1) *Haemonchus contortus*, (2) *Oesophagostomum columbianum*, (3) Various species of *Trichostrongylus*, (4) *Strongyloides papillosus*.

When mixed cultures of these parasites were used, no difficulty was experienced in artificially producing a fatal panverminosis, but

the production of a progressive disease in sheep by infecting these with pure cultures of these parasites was found to be altogether a different matter. This was especially the case when the sheep were stabled and properly fed on a well balanced ration. There are quite a number of factors which may, to a greater or lesser degree, be responsible for this, viz.:—

(1) Under field conditions the worm infestation is usually a mixed one, and it seems that the animal is less able to withstand the onslaught of an attack at several different portions of the gastro-intestinal tract;

(2) Where the grazing is poor, particularly in quality, the parasites thrive exceedingly at the expense of the host;

(3) Exercise seems to be detrimental. This is especially evident in those areas where no jackal-proof fencing has been carried out, for it becomes necessary, in order to protect the sheep against the ravages of this vermin, to drive them to the shelter of kraals every night. This increases the amount of daily exercise of these animals, with the result that the resistance to the parasites is decreased, enabling them to bring about deleterious effects more rapidly, especially in those sheep in which the anaemia is already present;

(4) The method of infection is probably also of importance. Veglia (1918) believes that the larvae, in the case of natural infection in haemonchosis, pass into the rumen of sheep with the solid food, whereas when 10 c.c. of saline suspension of larvae are injected into the mouth, the larvae may pass straight into the abomasum with this liquid and are in these circumstances liable to pass into the intestine without having had time to undergo those changes which enable them to remain in the abomasum.

It was only after many disappointments and with a great deal of difficulty that a sufficient number of typical, fatal cases of pure haemonchosis were artificially produced for haematological study. The work on the haematology and pathology of Oesophagostomiasis and Trichostrongylosis, will be discussed in later papers. In connection with Strongyloides papillosus, no haematological observations will be recorded (numerous observations were actually made) as in not a single instance was a progressive disease produced, in spite of the fact that many of the animals infected passed faeces from which strong colonies were obtained by faeces cultures.

Throughout these experiments, all the animals, including the controls, were kept under identical conditions of housing, feeding and watering. No special attempt was made to draw the blood at definite intervals before or after watering. It was thought that the most uniform results would be obtained if the amount of water that the animals consumed was allowed to be regulated by the natural development of thirst. In order to achieve this, a plentiful supply of water was made always available. On this account it is believed that no undue concentration of the blood could have occurred at any time as a result of diminished ingestion of water.

TECHNIQUE.

The technique developed by Neser (1923) and exhaustively discussed by him in his work on " The Blood of Equines " is with few modifications applicable to haematological work in sheep. The methods of examination employed are exactly those advocated by Neser, except where such modifications were found to be necessary, and details of the latter only will be discussed. For the other details Neser's work should be consulted.

Collection of Blood.

It is obvious that a direct examination of the whole blood would give the most accurate absolute results concerning the morphology and other attributes of both the erythrocytes and the leucocytes, but where a systematic examination of the blood of a number of sheep had to be completed in one day, it was clearly impossible to make determinations on the whole blood.

Rossdale (1923) compares the haematocrit results obtained when working with whole blood and citrated blood. Solid crystals were used, as well as definite proportions of citrate solutions, for which a correction was made afterwards. In a number of cases investigated, he found the greatest deviation from the mean volume index to be 4 per cent. in the case of whole blood, 11 per cent. in the case of citrate crystals, and 14 per cent. where citrate solution was employed. He concludes that the addition to the blood of citrate or oxalate affects the haematocrit readings by reducing the percentage volume of red cells, unless that particular proportion of citrate to blood be chanced upon which happens to be isotonic with the particular blood under investigation. Rossdale gives a comprehensive review of anticoagulants used by various workers, and he quotes in each case the haematocrit values obtained by these authors when centrifuging at certain definite speeds samples of blood of which the red counts, and in some cases average measurements of the red cells had been determined.

The anticoagulant selected was the one used by Neser for horse blood. This consisted of a 7½ per cent. citrate solution used in the proportion of .1 c.c. citrate to 1 c.c. of blood. With this method quite uniform relative results were obtained. Bryuchonenko and Steppuhn (1927) claim that Bayer 205, when injected intravenously, will prevent coagulation of the blood for some hours. This would be an ideal method for haematological work, provided the drug has no specific effect on any of the elements of the blood. The chief advantages of this method of collecting the blood would be: (1) If coagulation is prevented for some hours, there is ample time for a complete systematic study of several samples. (2) No measured amount of blood need be drawn. Errors in haematological studies are frequently due to the inaccurate measuring of the blood when it is being withdrawn from the vein. (3) No changes in isotonicity can possibly occur, so that values dependent on the size of the red cells will be more reliable. Hirudin as an anticoagulant was not available.

When an anticoagulant is used, the possibility of error is less the larger the amount of blood that is drawn. Where, however,

anaemia is studied, particularly in a small animal like the sheep, it is advisable that only small amounts of blood be collected, so that the factor of bleeding cannot be blamed for the production of the anaemia; consequently small bottles with narrow necks, measuring approximately 6 c.c. were utilized for collecting the blood.

Methods of Examination of the Blood.

The examination of the blood included the study of (1) the erythrocytes—(*a*) the red counts, (*b*) the red precipitate or the percentage volume of the red cells, (*c*) the percentage of haemoglobin, (*d*) the size of the red cells, (*e*) the viscosity. (2) The leucocytes—(*a*) the total white counts, and (*b*) the differential counts.

(1) (*a*) *Counting of the Red Cells.*

This was done in the usual way, using a Burker Turk counting chamber.

(1) (*b*) *The Determination of the Percentage Volume of Red Cells (Red Precipitate).*

This was obtained by centrifuging the citrated blood in an electric centrifuge running at 1,500 revolutions per minute, for 60 minutes. The centrifuge tubes were calibrated as advocated by Neser (1923). In the horse this author obtained constant centrifuge readings in 10 minutes, irrespective of whether the centrifuge was running at medium or high speed. At a low speed (250 revolutions per minute), the same reading was obtained in 15 minutes. Neser concluded that within certain limits of speed the volume of the red corpuscles in equine blood is in no way influenced by the speed.

The blood of the sheep, as compared with that of the horse, takes a much longer time to precipitate completely, as will be seen from the following experiment.

A young healthy Merino sheep, No. 24197, was bled daily from the jugular vein, 200-300 c.c. of blood being removed at a time, until a severe degree of anaemia was produced. Before bleeding, this animal had a red count of 15 million per c.mm. of blood, a percentage volume of 35, and 102 per cent. of haemoglobin. As a result of the bleeding, the red cell counts, the percentage of haemoglobin and the red precipitate rapidly decreased. When the sheep had developed what could be regarded as a mild degree of anaemia, with a red count of 4.9 millions per c.mm., a haemoglobin of 45 per cent. and a red precipitate of 15, the precipitate during centrifugalization was read every 10 minutes and the blood from another but normal sheep, No. 24188, was treated in the same way, at the same time, and in the same centrifuge running at 1,500 revolutions per minute. The results obtained were:—

Sheep No. 24197 (mild anaemia).					Sheep No. 24188 (normal).
ppt.	after	10	minutes	20	50
,,	,,	20	,,	18	34
,,	,,	30	,,	16	31
,,	,,	40	,,	15.5	30
,,	,,	50	,,	15	29.5
,,	,,	60	,,	15	29
,,	,,	70	,,	15	29
,,	,,	80	,,	15	29

Two days later, when a more severe degree of anaemia had developed, with a red count of 2.7 million per c.mm., the precipitate was again read at intervals of 10 minutes, together with that of the same normal sheep. The results obtained were:—

Sheep No. 24197 (severe anaemia).					Sheep No. 24188 (normal).
ppt. after		10	minutes	10	45
,,	,,	20	,,	9.5	38
,,	,,	30	,,	9	35
,,	,,	40	,,	9	34
,,	,,	50	,,	9	32
,,	,,	60	,,	9	31
,,	,,	70	,,	9	30
,,	,,	80	,,	9	30
,,	,,	90	,,	9	30
,,	,,	100	,,	9	30

From the above it can be concluded that in normal sheep constant red cell percentage volume readings are obtained in 60-70 minutes, when citrated blood is precipitated in an electric centrifuge running at 1,500 revolutions per minute. In the case of anaemic blood, constant readings are obtained very much earlier. In very severe degrees of anaemia, constant readings are obtained in 20-30 minutes time and in less severe degrees of anaemia in 40-50 minutes. In this work, therefore, the precipitate was read as a routine procedure after 60 minutes with the centrifuge running at 1,500 revolutions per minute.

(1) (*c*) *The Determination of the Percentage of Haemoglobin.*

This was determined calorimetrically in a Dubosq calorimeter, a Newcomer disc being used as the standard. The application of this disc to haemoglobin determinations was discussed by Newcomer in 1919. Daylight illumination was used as it was found difficult to match the colours with the artificial light available. Occasionally, when observations were made on cloudy days, it was found difficult to obtain consistent readings. Throughout this work an average of 10 calometric readings was taken, typical examples of which are:—

Sheep No. 11806—normal animal—dilution 1 in 200—8.2, 8.3, 8.2, 8.3, 8.2, 8.2, 8.2, 8.2, 8.2, 8.4—giving an average reading of 8.2. When this is read against the chart supplied by the manufacturers, the value of 92 per cent. haemoglobin is obtained.

Sheep No. 14197—showing anaemia—dilution 1 in 200—17.6, 17.7, 17.7, 17.8, 18.0, 17.9, 18.0, 17.6, 17.6, 17.9—giving an average of 17.8, which is equivalent to 42 per cent. of haemoglobin.

The Newcomer disc was standardized against Van Slyke's gasometric method, and the chart supplied by the manufacturers of the disc in the Williamson standard was converted into the Haldane standard. Newcomer (1923) described "a new optical instrument for the determination of haemoglobin." This should be a very convenient apparatus, as one is able to read off directly on the instrument the percentage of haemoglobin.

The diluting fluid is N/10 hydrochloric acid which converts the blood into acid haematin. The chart supplied with the disc is only

prepared for reading dilutions of 1 in 200 and 1 in 500. In this work the dilution of 1 in 200 was employed.

Newcomer (1923) points out it takes some time before the full colour depth of the acid haematin is developed. After the flasks have stood for half an hour, the readings are correct to approximately 1 per cent. Readings were never made within an hour after making the dilutions, but some readings were not made until some hours after dilution. If it is necessary to determine the haemoglobin content almost immediately after making the dilutons, the readings should be corrected in accordance with Newcomer's (1919 and 1923) equation.

Terrill (1922) discusses (1) a method for making concentrated stock solutions of acid haematin. When these are kept in the dark at room temperature, or in a refrigerator, the colour fades slowly. He does not mention to what extent fading occurs if the stock solution be exposed to light. (2) A method for the preparation of acid haematin protein powder, from which standard solutions are prepared. (3) A method of preparing acid haematin films on cover glasses by means of a mixture of solutions of acid haematin and of gelatin. This author in the same paper shows that turbidity in the acid haematin solutions can be got rid of, by means of laking the blood with distilled water, before adding it to the hydrochloric acid solution. He maintains that the whole blood diluted with N/10 hydrochloric acid gives reading 6-12 per cent. higher than those obtained for the same blood first laked by adding it to distilled water. This error should not occur in the figures obtained in this investigation as the disc was standardized against Van Slyke, by obtaining the average of a number of calorimetric readings by different individuals on an acid haematin prepared without previous laking.

There is one important point to bear in mind when making the dilutions, viz., to shake the citrated blood very thoroughly before it is drawn off with the pipette for making the dilutions. If this is not done, sedimentation may occur and proportionately more red corpuscles are drawn into the pipette, which naturally is conveniently rested against the bottom of the bottle during manipulations. A considerable source of error in all the determinations may be introduced if this point is neglected. It is also important to avoid the formation of bubbles in the pipette, as well as in the flasks in which the acid haematin dilutions are made. Such bubbles are especially liable to form in the pipette on top of blood withdrawn for the first time from a bottle which just previously has been vigorously shaken. When this is the case the blood should again be blown into the bottle, whilst holding the pipette slightly obliquely and resting it against the bottom of the bottle. No bubbles will be present when the blood is sucked up the second time. In order to avoid bubbles in the diluting flask, the blood should be run down the side of the flask and should not be vigorously squirted into the N/10 hydrochloric acid solution. It was found convenient to fill the flask to within 10-20 cc. of the 200 cc. mark and then to run in the citrated blood as described. If the flask is now tilted or shaken, bubbles will develop, and it will be difficult to fill the flask to the mark. The flask should only be tilted after further N/10 hydrochloric acid has been carefully

added to the 200 cc. mark. Flasks with long necks, having the 200 cc. mark on the neck, are the most convenient. The 1 cc. pipettes used were either standard or previously checked against a standard pipette.

(1) (*d*) *Size of the Red Cells.*

The size of the red cells was determined by direct microscopic measurement with an "Okular Schraubenmikrometer," having a 6 compensating ocular. When this apparatus is used with a 2 mm. apochromatic lens, the figures obtained must be multiplied by .14 in order to convert them into values of microns. It is realized that the true average size of red cells in stained smears will not be obtained by measuring even 400 or more red cells in numerous fields, and as this is most exacting work, requiring a great deal of time, only relatively few cells could be measured, mainly with the object of obtaining corroborative evidence as to whether any increase at all in the size of the cells had occurred, as was suggested by changes in the colour index and in the volume index. Pyper (1929) describes a diffraction method whereby the true average size of the red cells in suitably prepared unstained smears can be simply and quickly determined spectroscopically. If a sufficient number of smaller and larger cells are present in such smears, they will also produce measurable spectra, consequently this author can by this method calculate the diameters of the smallest, the average, and the largest cells. The method was unfortunately not available for application in this study.

(1) (*c*) *The Viscosity.*

Hess' viscosimeter was used. Schridde and Naegeli (1921) give a detailed description of this apparatus. Unless the determinations are made with dexterity, the values obtained can be very unreliable. In the tables submitted, the temperatures at which determinations were made are stated, but no corrections for these were made. It is noteworthy that in some cases in which there was observed an apparent agglutination of red cells in the counting chamber, the viscosity was abnormally high for the particular blood, if it is considered that more or less normal values were obtained for the red counts, haemoglobin and percentage volume of red cells.

2. *Leucocytes.*

The total white cell counts were made by the usual methods and the differential counts were made according to Neser's (1923) modification of Ehrlich's method.

DETAILS OF EXPERIMENTS.

As already stated it was at first intended to make a comparative haematological study of sheep with pure infestations of *Haemonchus contortus*, various species of *Trichostrongylus*, *Oesophagostomum columbianum* and *Strongyloides papillosus*.

Seventy sheep were used in these experiments. Thirty-eight were infected with *Haemonchus contortus*, eleven with *Trichostrongylus*, seven with *Oesophagostomum columbianum*, three with

Strongyloides papillosus and eleven were kept as controls. In this work details in connection with the haematology and pathology of haemonchosis only will be presented.

THE HAEMATOLOGY AND PATHOLOGY OF HAEMONCHOSIS.

Sheep infected with a pure or practically pure culture of *Haemonchus contortus* can be conveniently classified in terms of the effects observed as: (A) those sheep in which a progressive and fatal disease was produced and (B) those in which an anaemia developed from which the animals soon recovered, without medicinal or any other treatment—these can be designated as recovery cases and those in which no effects were observed. (C) Controls.

(A) Changes Observed in the Blood of Sheep in Fatal Cases of Haemonchosis.

The outstanding feature of these cases is a progressive anaemia. This is conveniently illustrated in the accompanying tables and graphs, where the changes in the red cells are recorded in so far as are concerned (1) the number of red cells, (2) the percentage of haemoglobin, (3) the percentage volume of red cells (precipitate), and in some cases, (4) the viscosity.

The total number of leucocytes is also charted, together with differential counts, where the latter could conveniently be dealt with in this way.

Of the thirty-eight sheep infected artificially with *Haemonchus contortus*, nine developed a progressive anaemia, with a fatal termination. There were six so called recovery cases and the remaining twenty-three either resisted infestation or did not show any effects of infection. Of the nine cases in which a progressive disease was produced, the haematological observations of five will be presented in detail.

Haematology and Pathology of Sheep No. 16027.

Table 1 and graph 1 contain the haematological records of Sheep No. 16027. Infection was commenced on the 27.4.27, at the rate of approximately 1,000 larvae per day, until 52,000 larvae had been given. Infection was, therefore, completed towards 16.6.27.

Haematology of Sheep No. 16027.

During the first fourteen (14) days after infection there is an increase in the number of red cells, with a corresponding increase in the percentage of haemoglobin and in the percentage volume of red cells. Such increases during the initial stages of the infection period have been observed in quite a number of cases, and are interpreted as probably due to increased activity of the blood-forming organs. The destruction and removal of red cells during the early stages of *Haemonchus contortus* infection serve as the stimulus for this increased activity. After that there is a progressive decrease in (1) the number of red cells, (2) the percentage of haemoglobin, and (3) the percentage volume of red cells, except for a slight increase which

TABLE 1.

SHEEP No. 10627.

Method of treatment: Infection commenced 27.4.27, giving 1,000 *larvae of* Haemonchus contortus *per day until* 52,000 *larvae had been administered.*

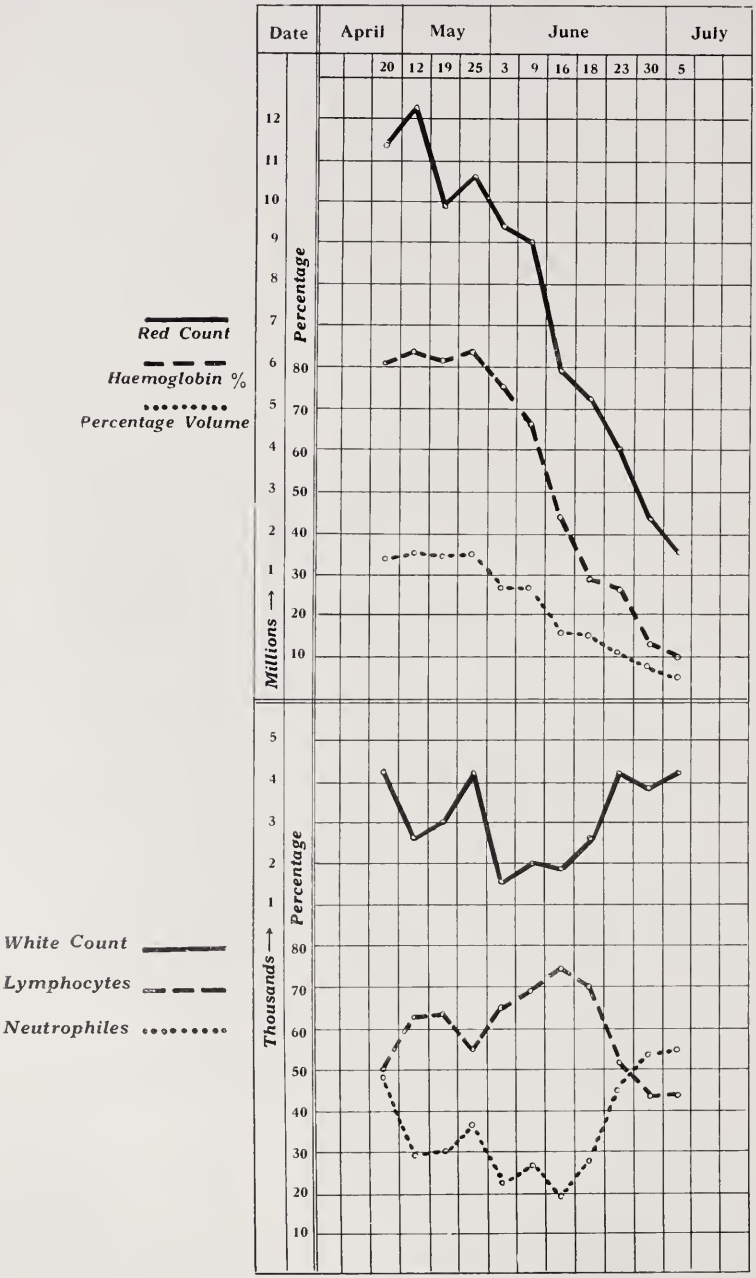
Date.	Source.	R.C.	R.P.	Hglb. %	Serum.	W.C.	L.	M.	N.	E.	B.	V.C.I.	C.I.	V.I.	Remarks.
20/4/27	Jugular	11·4	34	82*	Clear	4,200	50	1	49	0	0	1·00	0·94	0·95	Red cells normal.
12/5/27	,,	12·2	35	84	,,	2,700	63	4	29	3	1	0·99	0·91	0·92	Red cells normal.
19/5/27	,,	9·9	34	81*	,,	3,000	64	5	30	1	0	0·99	1·07	0·83	Red cells normal.
25/5/27	,,	10·6	35	83	,,	4,200	56	2	37	3	2	0·99	1·04	1·06	Red cells normal.
3/6/27	,,	9·4	27	76	,,	1,600	66	4	22	8	0	1·17	1·30	0·92	Red cells normal.
9/6/27	,,	9·0	27	67	,,	2,000	69	1	27	3	0	1·03	0·99	0·96	Red cells normal.
16/6/27	,,	5·9	16	43	,,	1,900	75	5	19	1	0	1·12	0·97	0·87	Anisocytosis with occasional punctate basophilia.
18/6/27	,,	5·3	15	29	,,	2,600	70	2	28	0	0	0·80	0·72	0·90	Anisocytosis with occasional punctate basophilia.
23/6/27	,,	4·0	11	27	,,	4,200	51	4	45	0	0	1·02	0·90	0·88	Polychromasia-anisocytosis punctate basophilia.
30/6/27	,,	2·3	8	13	,,	3,900	43	2	53	2	0	0·68	0·76	1·11	Marked anisocytosis, poikilocytosis, polychromasia, punctate basophilia.
5/7/27	,,	1·6	5±	10	,,	4,200	43	3	54	0	0	0·84	0·83	1·00	Marked anisocytosis, poikilocytosis, polychromasia, punctate basophilia. Excepting polychromasia less marked. Jolly bodies present. DIED.

* On these two days haemoglobin was not actually measured but was calculated.

Although infection only occurred on 27/4/27, the first four determinations, i.e., up to and including those on 25/5/27, are regarded as normal.

Abbreviations: R.C. = Red count. R.P. = Red precipitate. Hglb. = Haemoglobin. W.C. = White count. L. = Lymphocyte. M. = Monocyte. N. = Neutrophiles. E. = Eosinophiles. B. = Basophilia. V.C.I. = Volume colour index. C.I. = Colour index. V.I. = Volume index.

For method of calculating colour indices, see Table IV.



GRAPH 1.

SHEEP No. 1627.

Infected *Haemonchus contortus*
(52,000 larvae).

Killed by pure infection of *Haemonchus contortus* (Wireworm)

occurs during the period from the 19th until the 25th of May. From the 9th to the 16th of June the decrease of 3 million red cells per c.mm. of blood, of 24 per cent. in the haemoglobin, and of 11 per cent. in the percentage volume is particularly striking. During this time no clinical evidence of icterus or haemoglobinuria was present. The animal was destroyed on the 5th of July, on which day the red count was 1.6 million per c.cm. of blood, the haemoglobin 10.5 per cent. and the precipitate approximately 5 per cent.

During the course of the disease the only clinical symptoms observed were (1) pale mucous membranes, particularly the conjunctival, the buccal and the anal (just before death the pallor was of an extreme degree), (2) accelerated respirations, and (3) frequent pulse, with pounding heart action, especially in the later stages. The accelerated respirations and frequent heart action were particularly noticeable on exertion. There was no clinical evidence of icterus or oedema.

Changes in the Red Cells.—On referring to Table No. 1 it will be seen that no morphological changes occurred in the red cells until 16.6.27. On that day punctate basophilia was for the first time observed. During the further course of the disease, and as the anaemia became progressively worse, the morphological changes became more pronounced. On 30.6.27 and 5.7.27, when the anaemia was of an extreme degree, there was well marked anisocytosis, and there were poikilocytosis, polychromasia, punctate basophilia and Jolly bodies in quite a number of red cells, but in this particular case no normoblasts were identified.

Changes in Size of the Red Cells.—As already stated, Pyper's (1929) apparatus for determining quickly and simply the average size of the red cells in smears was not available. To measure say 400 cells with a micrometer requires a great deal of time and even if this is done the true average size of the red cells in smears will not be obtained with any degree of accuracy. It is, however, important for the interpretation of changes in colour and volume indices, to know if changes in size have occurred. In this particular sheep measurements were made of a small number of cells from smears made (1) before infection, (2) when a marked decrease in the oxygen carrying capacity of the blood was taking place, and (3) towards the end of the disease. In all three cases 15 cells were measured in several fields. The results are:—

Date.	*Smallest cell.*	*Largest cell.*	*Average.*
(1) 20.4.27	4.17μ	5.2μ	4.6μ
(2) 16.6.27	3.9μ	5.3μ	4.6μ
(3) 30.6.27	4.2μ	7.1μ	5.7μ

The relationship of the haemoglobin content to the number of red cells, i.e., the colour index, the relationship of the haemoglobin to the precipitate, i.e. the volume colour index, and the relationship of the precipitate to the number of red cells, i.e. the volume index, will be fully discussed elsewhere in this paper. This being the case, it will only be necessary to indicate briefly and without going into any details the more important changes that have occurred.

The Colour Index.—There is a tendency towards a decrease of this index, probably due to a relative decrease in the haemoglobin content of the cells and to an increase in the size of the cells.

The Volume Colour Index.—There is at first a tendency towards an increase of this index (up to 16.6.27) probably due to a decrease in the size of the red cells. Towards the end of the disease the index is definitely decreased. This would indicate a relative deficiency in haemoglobin and an increase in the size of the red cells.

Volume Index.—There is at first a tendency towards a decrease of this index, indicating a decrease in the size of the red cells, but towards the end of the disease the volume index increases. This confirms the conclusion previously arrived at, that an increase in the size of the red cells had occurred. As previously stated, too few cells were measured in order that reliable conclusions as to the true average size of the red cells could be drawn; nevertheless, the measurements made suggest that the cells on 16.6.27, for instance, are on the whole smaller than the cells on 20.4.27. There is no doubt at all that on 30.6.27 the cells were definitely larger than before infection and during the earlier stages of the disease.

The cells stained less intensely in the later stages of the disease and many had a vacuolated appearance. Although unstained central areas are sometimes encountered in cells of apparently normal sheep, these staining reactions would tend to confirm the conclusion that a relative deficiency of haemoglobin was present.

Changes in the Leucocytes.—No changes which can be regarded as pathognomic for haemonchosis were observed. Soon after the red cells begin to decrease in number a decrease in the total number of leucocytes also occurs, but during the later stages of the disease the total number of leucocytes again increases. If the differential counts be referred to, it will be observed that this increase affects mainly the neutrophiles. The neutrophile counts are at their lowest when the first evidence of morphological changes in the red cells is noticed. Later on, when these morphological changes in the red cells are more pronounced, the neutrophiles increase in number. It is possible that this may be associated with greater activity of the blood-forming organs. No marked changes were observed in connection with the number of eosinophiles, only on one day (3.6.27), during the early stages of the disease, was there what one could describe as an eosinophilia.

Pathology of Sheep No. 16027.

On 5.7.27 the animal was killed *in extremis* for post mortem examination.

Macroscopic Examination.—Signalment: Merino wether in good condition, having only temporory teeth.

The mucous membranes of the natural openings were very pale and almost dead white in colour. The *blood* was extremely watery, barely staining the fingers, and was of almost an amber colour. A good deal of fat was present in the fat depôts. No abnormal fluid was present in the abdominal cavity. The *lymphatic glands* on section were pale and moist. The pericardial sac contained about

20 c.c. of a clear colourless fluid. The *liver* was slightly enlarged and firm on palpation. On section a watery fluid escaped from the cut surface, which was of a mottled appearance, the central portion of the lobule being brown and opaque and the periphery almost transparent and grey in colour. The heart fat was normal in amount and appearance. The *heart* itself appeared quite normal, except that the myocardium was of a greyish colour and opaque, suggesting degenerative changes of a fatty nature. The *lungs* were extremely pale, but excepting for a certain amount of emphysema, were not altered in any way. The *kidney* had a fair amount of pericapsular fat, the capsule stripped easily, the cortex was of a yellowish brown colour and the medulla and boundary zones were extremely pale. The *spleen* was decreased in size and measured 7.5 by 5 by 1 cm. The capsule was markedly wrinkled, the trabeculae were distinct, but the Malpighian bodies were indistinct. The other organs, such as the *adrenals*, *thyroid*, etc., were all systematically examined, but apart from the fact that they were paler in colour than normal, no abnormalities were observed macroscopically. The contents as well as the mucous membranes of the *rumen*, *reticulum* and *omasum* were normal. In the *abomasum* numerous wire-worms were present The mucous membrane was pale and the contents were liquid and of a dark brownish colour. In the *small intestine* the ingesta were liquid and of a dark chocolate colour and a few *Strongyloides papillosus* were present. The faeces in the large intestine were of a dark colour. Very few adults of the *Oesophagostomum columbianum* were identified, but no nodules were present. No changes were recognized in the bone-marrow.

Anatomical Pathological Diagnosis.—Very marked anaemia, paleness of all the organs and of the visible and other mucous membranes, throughout the body; degenerative changes of the liver, myocardium and kidneys; emphysema of the lung; numerous wire-worms; very few nodular worms; few *Strongyloides papillosus;* complete absence of icterus and haemoglobinuria.

Aetiological Diagnosis.—Killed in extremis (practically pure infection of *Haemonchus contortus*).

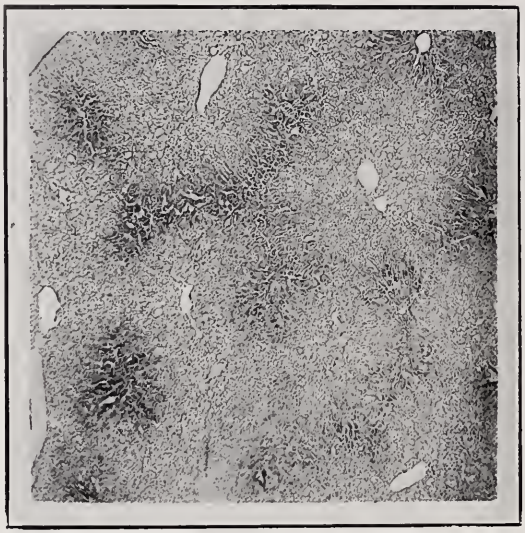
Microscopic Examination: *Kidneys.*—There were slight degenerative changes of a fatty nature. Some of the cells, especially of the convoluted tubules, stained a faded orange with Sudan III, and with a 4 mm. objective were seen to be distended with fine radiating crystals, which stained similarly. There was no evidence of any iron-containing pigment in the kidney. No other lesions were present.

Spleen and Lung.—No changes were seen microscopically, except that the lymphoid follicles in the spleen were not well defined, and emphysema of lungs was present. With specific staining for iron, very little iron-containing pigment could be identified in the spleen and none at all could be recognized in the lungs.

Liver.—When the haemalum-eosin stained sections were examined with the naked eye by holding them up against the light, a distinct mottled appearance was observed. This was due to some portions staining slightly or not at all and other staining more evenly and more intensely (see Figs. 1 and 2.) On microscopic examination the slightly stained or even unstained areas were seen to be

SHEEP No. 16027: SPEC. No. 7045.

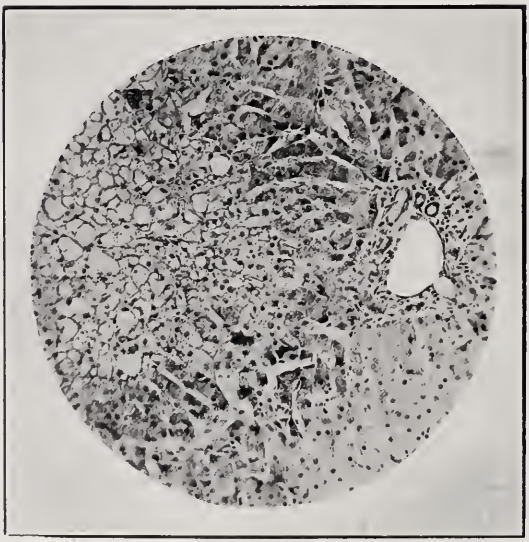
FIG. 1.



Liver. ×33.

Lesions around Central Veins.

FIG. 2.



Liver. ×110.

Lesions around Central Veins.

SHEEP No. 16027: SPEC. No. 7045.
FIG. 3.

Liver. ×450.

Crystals in Liver Cells.

SPEC. No. 11038.
FIG. 4.

Fat Necrosis in Sheep. ×225.

those situated around the central veins. Here the cells contained large vacuoles, which did not stain at all, but the peripheries of the cells were well defined as slender pink staining outlines. Many cells were devoid of a nucleus and in others the nucleus was situated against the periphery of the cytoplasm, although cells having a more centrally situated nucleus were also present. Towards the periphery of the lobule, i.e. around Glisson's capsule, the cells were intact and normal in appearance. The extent to which the cells with these vacuoles occurred varied. The greater proportion of some lobules were so affected, with the result that there remained only a narrow peripheral portion of the lobule, with more or less normally staining liver cells.

This vacuolated appearance of the cells was also seen to be present in van Gieson and Giemsa stained sections, but with Sudan III the contents of these cells stained in most cases a faded orange colour. A large number of cells was distended with fine radiating crystals (see Fig. 3) somewhat like those seen in fat necrosis (see Fig. 4). These crystals also stained a faded orange colour with Sudan III. It was only here and there that cells containing true fat were encountered. The presence of a very little true fat was confirmed when sections stained with Nile Blue Sulphate were examined. With this stain, some of the cells around the central veins stained blue and others remained unstained. The crystals previously referred to were for the most part unstained, although some did stain a light blue. According to Mallory and Wright (1922), such colour reactions are obtained with soaps, fatty acids and lipochromes. The interpretation of these lesions will be fully discussed elsewhere. No demonstrable iron containing pigment was present in the liver.

These peculiarly staining crystals were found in epithelial cells of nearly all the organs including the lung, thyroid, intestine and adrenals. In the case of the latter organs only the cells containing these crystals stained with Sudan III. No crystals were, however, demonstrable in the myocardium.

Haematology and Pathology of Sheep No. 11896.

Table 2 and graph 2 contain the haematological records of Sheep No. 11896. Infection was commenced on 16.11,25 at the rate of 200 larvae every second day until 600 larvae had been given. Thereafter 5,000 larvae were given every second or third day until approximately 50,600 larvae had been given. The last 5,000 larvae were given on 23.12.25.

Haematology of Sheep No. 11896.

Changes in the red cells.—Very soon after infection the number of red cells begins to decrease. This is also reflected as a decrease in the percentage of haemoglobin and the red precipitate. By 20.11.25 600 larvae had been administered (200 on 16/11, 200 on 18/11 and 200 on 20/11). During the following 5 days a remarkable drop of more than 3 million red cells per c.mm. of blood occurred and the question which immediately arises here is whether this marked decrease of the number of red cells is or is not entirely due to the action of the parasites. This is very difficult to prove, and one

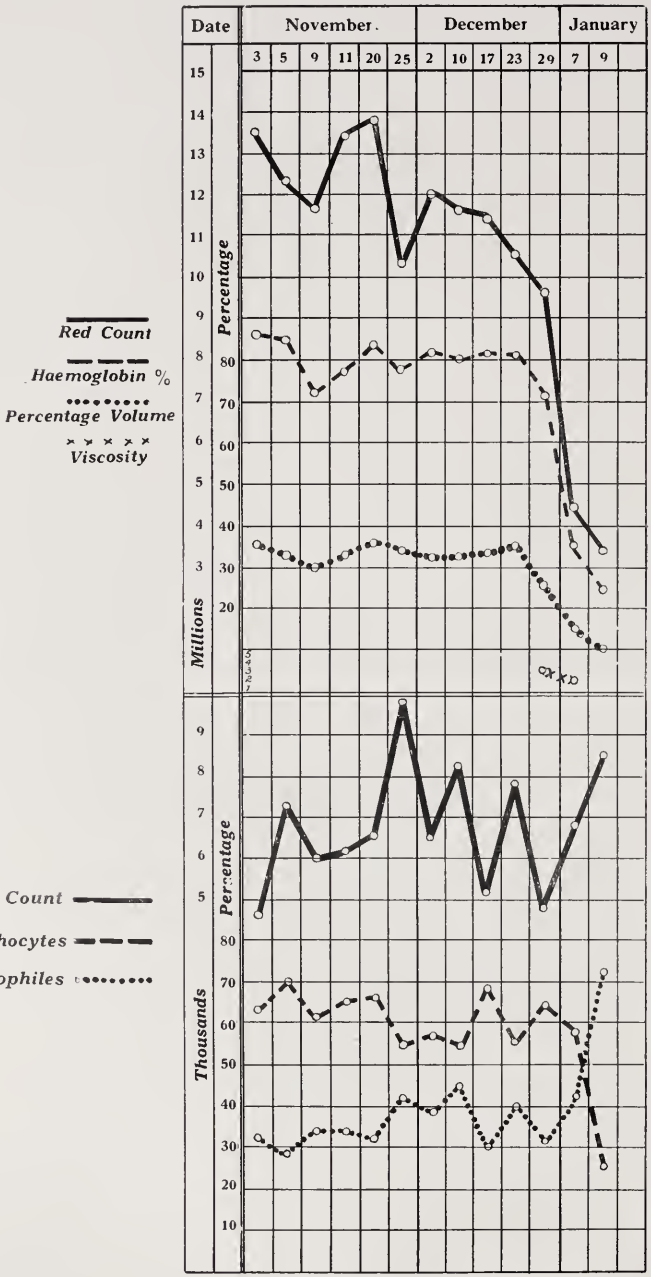
TABLE 2.

SHEEP No. 11896.

Method of treatment: Infection commenced 16.11.25 *with* Haemonchus contortus *and continued every second or third day until* 50,600 *larvae had been given. Infection completed by* 23.12.25.

Date.	Source.	R.C.	R.P.	Hglb. %	Serum.	W.C.	L.	M.	N.	E.	B.	V.C.I.	C.I.	V.I.	Remarks.
3/11/25	Jugular	13·5	36	86	Clear	4,600	63	5	31	1	0	1·00	0·98	0·99	Red cells normal.
5/11/25	,,	12·3	34	84	,,	7,200	70	0	29	1	0	1·03	1·06	1·03	Red cells normal.
9/11/25	,,	11·7	30	72	,,	6,000	61	3	34	2	0	1·00	0·96	0·95	Red cells normal.
11/11/25	,,	13·4	33	78	,,	6,100	65	0	34	1	0	0·99	0·91	0·91	Red cells normal.
20/11/25	,,	13·9	36	83	,,	6,500	66	1	32	1	0	0·96	0·93	0·96	Red cells normal.
25/11/25	,,	10·3	34	78	,,	9,800	54	1	42	2	1	0·96	1·18	1·22	Red cells normal.
2/12/25	,,	12·0	33	81	,,	6,400	57	2	39	1	1	1·03	1·05	1·02	Red cells normal.
10/12/25	,,	11·7	33	80	,,	8,200	55	0	45	0	0	1·05	1·10	1·04	Red cells normal.
17/12/25	,,	11·4	33	81	,,	5,100	69	0	30	1	0	1·03	1·11	1·04	Red cells normal.
23/12/25	,,	10·5	35	81	,,	7,900	56	2	40	2	0	0·97	1·20	1·23	Red cells normal.
29/12/25	,,	9·6	26	70	,,	4,800	64	2	31	3	0	1·13	1·13	1·00	Red cells normal.
7/1/26	,,	4·4	15	34	,,	6,900	58	0	42	0	0	0·95	1·20	1·26	Anysocytosis. Suggestion of polychromasia. Occasional Jolly bodies.
9/1/26	,,	3·3	10	23	,,	8,400	26	2	72	0	0	0·96	1·08	1·12	Anysocytosis. Suggestion of polychromasia. Occasional Jolly bodies.

Although infection was commenced on 16/11/25, the first seven determinations, i.e., up to and including 2/12/25, are regarded as normal.
For abbreviations see Table I.
For method of calculating colour indices, see Table IV.



GRAPH 2.
SHEEP No. 11896.
Infected *Haemonchus contortus*
(50,600 larvae).
Killed by pure infection of *Haemonchus contortus* (Wireworm).

hesitates to draw that conclusion, particularly in view of the fact that infection was commenced only 9 days previously. According to Veglia (1915) the larvae that were administered on 16.11.25 would be, on 25.11.25, just on the point of undergoing their 4th ecdysis before entering on the adult stage. In the same paper Veglia describes blood coagula in the mucous membrane of the abomasum, 3 days after infection. In the first parasitic stage of *Haemonchus* larvae, the mouth parts are not armed with a lancet (which only develops during the 2nd parasitic stage and apparently only comes into use in the adult stage), but the larvae are already able to produce in the 2nd parasitic stage, i.e. the 4th stage larvae, the blood coagula described by Veglia. It seems, therefore, possible that these young forms can produce changes in the blood, but it is doubtful if this is likely to occur to the extent of decreasing the red counts by 3 million per c.mm. of blood in 5 days (from 20.11.25-25.11.25). That the effects produced by them must be regarded as a definite contributory cause of this is without question, but a number of other factors may also be partly responsible for this big decrease in the red counts. If the blood volume had become increased such low counts might have been obtained. On the other hand, the blood might have been unduly concentrated on 11.11.25 and on 20.11.25. This could have occurred under certain abnormally dry conditions and high temperatures of the atmosphere, or if the animal incidentally had a diarrhoea or polyuria. No observations to obtain information concerning these several factors were made at the time. If there was overconcentration of the blood, the true counts with a normal blood volume may really have been somewhere in the neighbourhood of 12 million cells (instead of 13.9 million, as actually recorded on 20.11.25). This decrease would then have been correspondingly less.

From 2.12.25 there is a continuous and progressive decrease in the number of red cells every week. During the second week after the administration of the last dose of 5,000 larvae, there is practically a collapse of the red count, and this decrease by more than 5 million red cells per c.mm. of blood during 9 days time, must be regarded as phenomenal. It does seem rather paradoxical that the anaemia develops progressively more rapidly when the animal is no longer being infected. It may of course be that just about this time the limits of compensation were being reached, and that, if infection had been continued, a similar or even greater collapse would have occurred. Did this not occur with continuous administration of infective larvae, then it would appear that the method of infection employed was wrong. Instead of giving large doses at frequent intervals, a single large dose, or smaller doses at greater intervals, should perhaps have been given. When large doses are given at short intervals disturbances in the abomasum may be brought about, as a result of which a large proportion of the infection may become lost. This may actually be responsible for the so-called recovery cases.

It will be observed that the progressive decrease in the number of red cells which occurred from 2.12.25 to 23.12.25 is not reflected in the haemoglobin and percentage volume curves. The haemoglobin remains more or less stationary, but the precipitate is even somewhat increased on 23.12.25. If one could at this time have obtained reliable data of the true average size of the red cells, a satisfactory

explanation of these results would probably have been obtained. Measurements that were made indicate that there was an increase in the size of the cells, but it is realized, as previously stated, that too few cells were measured in order that reliable conclusions could be drawn in this connection. It is nevertheless of interest to present the figures that were obtained. These comprise measurements that were made during the pre-infection period, and at various intervals during the course of the disease, 15 cells being measured in various fields in each case. The results are tabulated below:—

Sheep No.	*Date.*	*Minimum.*	*Maximum.*	*Average.*
11896	9.11.25	4.1μ	5.0μ	4.3μ
,,	23.12.25	3.8μ	4.8μ	4.5μ
,,	7.1.26	4.0μ	5.2μ	4.9μ
,,	9.1.26	3.9μ	5.4μ	4.6μ

Changes in colour and volume indices.—On referring to Table 2, it will be seen that there is an increase in the volume index and in the colour index, but there is a decrease in the colour volume index. These changes in the indices can be caused by an increase in the size of the red cells.

On 7.1.26 and 9.1.26 morphological changes, such as anisocytosis, a suggestion of polychromania and occasional Jolly bodies were recorded, but no other evidence of anaemia was found on microscopic examination.

Changes in the Leucocytes.—A leucocytosis was present on 25.11.25, i.e. about 9 days after infection was commenced, and a neutrophile leucocytosis was recorded towards the end of the disease.

Pathology of Sheep No. 11896.

(1) *Macroscopic Examination.*—This merino ewe lamb died on 11.1.26. In presenting the results of the post-mortem examination, it is not considered necessary to give a detailed description of all the organs examined. Thus reference need only be made to organs in which changes were recognized.

The blood was coagulated. There was marked anaemia with paleness of all the organs and of the visible and other mucous membranes. There were degenerative changes in the heart and the liver and slight oedema of the lungs. The abomasal contents were of a yellowish chocolate colour. Wireworms were exceedingly numerous. A few nodules of *Oesophagostomum columbianum* were present in the small intestine, but there were no adult nodular worms. The spleen showed slight atrophy, measuring 10 by 6.5 by 1.5 cm. No other parasites were present.

(2) *Anatomical Pathological Diagnosis.*—This was similar to that of sheep 16027, except that no parasites other than *Haemonchus contortus* were recorded.

(3) *Aetiological Diagnosis.*—Anaemia due to a pure infection of *Haemonchus contortus*.

(4) *Microscopic Examination.*—The lesions in all the organs were similar to those described for Sheep No. 16027, except in the case of the liver. In this organ the orderly arrangement of the cells around

the central veins was disturbed. The nuclei of these cells were pycnotic, and in some cases absent altogether. The cytoplasm of the cells stained more intensively with haemalumeosin than that of normal cells. These changes in the cytoplasm and the nuclei of the cells immediately around the central veins were interpreted as early stages of necrosis. Only occasional cells, some of which contained crystals similar to those previously described, stained with Sudan III. The staining reactions were otherwise similar to those described for Sheep No. 16027.

Haematology and Pathology of Sheep No. 11921.

Table 3 and graph 3 contain the haematological records of Sheep No. 11921. Infection with *Haemonchus contortus* larvae was commenced on 16.11.25, and continued every 2nd or 3rd day until 20,600 larvae had been administered. In the beginning each dose consisted of 200 larvae. The last of 3 such doses was given on 20.11.25. From 2.12.25 each dose consisted of 2,000 larvae. On 23.12.25 infection was completed.

Haematology of Sheep No. 11921.

Changes in the red cells.—During the pre-infection period, there is a certain amount of what is interpreted as normal variations in the red counts and other determinations that were made. During the infection period, there is even greater fluctuation in the counts, etc., but during this time there is a tendency towards an increase in the red counts, accompanied by a corresponding increase in the amount of haemoglobin and in the percentage volume of red cells. One is very much tempted to explain this as being due to increased activity on the part of the blood-forming organs, as a direct result of the action of the parasites. No demonstrable evidence of such activity could, however, be found when making careful microscopic examinations of stained smears during this time.

It is again somewhat significant that, within 14 days after infection was completed, the progressive decrease in the number of red cells commenced, and again the remarkable decrease of 5 million red cells per c.mm. of blood was registered within a period of 8 days. The percentage of haemoglobin is the lowest, and the percentage volume of red cells least, on the 3rd and 5th of February, 1926. On these dates, however, there is a slight increase in the red counts. There are quite a number of factors that may be responsible for this: (1) Error in counting: this is possible, and it is generally admitted that one of the most frequent sources of error in haematological work is in connection with the counting of the red cells. (2) Poikilocytosis and anisocytosis was of an extreme degree on these dates; microcytes were very numerous; macrocytes were less frequent. The anisocytosis is well shown in Fig. 6 (from smears made on 21.1.26), and should be compared with the normal cells of more or less uniform size, as seen in Fig. 5 (from smears made on 3.12.25). Fig. 6 is a microphotograph of cells from smears made on 21.1.26, when the red counts fell from 10.3 million of the previous week to 5.5 million per c.mm. of blood. The values obtained when measuring 15 cells from smears made on 3.12.25 are: 34.5, 33.5, 33.5, 31.0, 32.0, 34.5, 33.5, 31.5, 34.0, 31.5, 32.5, 36.0, 31.0, 32.5, 33.0, when the "Okular Schraubenmikrometer," having a 6 comp. ocular, with a 2 mm. apochromatic objective

TABLE 3.

SHEEP No. 11921.

Method of treatment: Infection with Haemonchus contortus *commenced on* 16.11.25 *and continued every second or third day until* 20,600 *larvae had been given.*

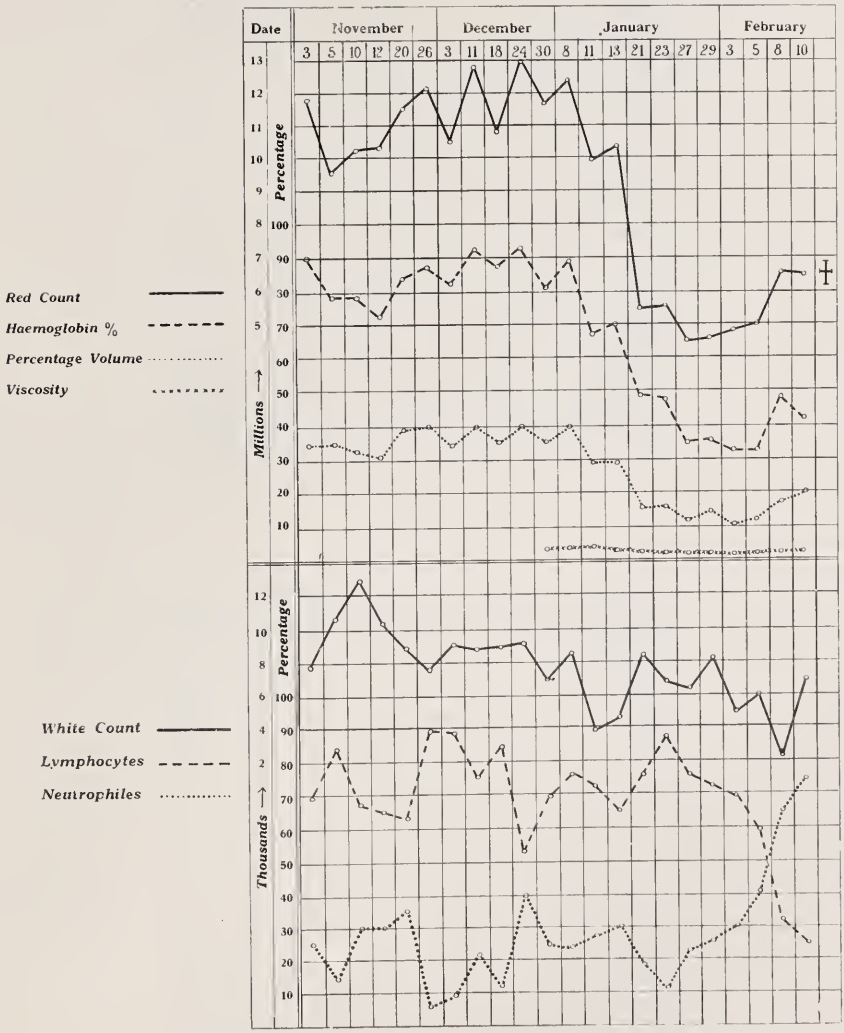
Date.	Source.	R.C.	R.P.	Hglb. %	Viscosity.	W.C.	L.	M.	N.	E.	B.	V.C.I.	C.I.	V.I.	Remarks.
3/11/25	Jugular	11·8	35	90	—	7,800	69	5	26	0	0	1·11	1·01	—	—
5/11/25	,,	9·6	35	78	—	10,500	84	0	14	1	1	0·96	0·92	—	—
10/11/25	,,	10·2	33	78	—	12,700	67	3	30	0	0	1·02	0·98	—	—
12/11/25	,,	10·3	31	72	—	10,300	65	4	30	1	0	1·01	1·08	—	—
20/11/25	,,	11·6	39	84	—	9,000	63	1	36	0	0	0·93	0·96	—	—
26/11/25	,,	12·1	40	87	—	7,800	89	5	6	0	0	0·90	0·95	—	—
3/12/25	,,	10·5	34	83	—	9,100	88	3	9	0	0	1·05	1·04	—	—
11/12/25	,,	12·8	40	92	—	8,900	75	3	21	0	1	0·99	0·96	—	Cells normal.
18/12/25	,,	10·8	35	87	—	9,100	84	2	12	1	1	1·08	1·07	—	Cells normal.
24/12/25	,,	13·0	40	93	—	9,300	53	6	40	1	0	1·01	0·95	—	Cells norma.
30/12/25	,,	11·7	35	81	3·5	7,000	69	4	24	3	0	1·06	0·92	—	Cells normal.
8/1/26	,,	12·4	40	89	3·6	8,300	76	1	23	0	0	0·91	0·95	—	Cells normal.
11/1/26	,,	9·9	29	67	3·9	3,900	72	1	27	0	0	1·00	0·90	—	Cells normal.
13/1/26	,,	10·3	29	70	3·0	4,500	66	3	30	0	1	1·05	0·90	—	Cells normal.
21/1/26	,,	5·5	16	48	2·3	8,400	76	4	18	1	1	1·29	1·16	—	Anisocytosis well marked.
23/1/26	,,	5·6	16	47	2·2	6,900	87	2	11	0	0	1·27	1·11	—	Anisocytosis well marked.
27/1/26	,,	4·5	12	35	2·0	6,300	76	0	23	1	0	1·26	1·03	—	Anisocytosis and poikilocytosis.
29/1/26	,,	4·6	14	36	2·0	8,200	72	1	26	0	1	1·11	1·04	—	Anisocytosis and poikilocytosis.
3/2/26	,,	4·8	11	33	1·6	5,000	69	1	30	0	0	1·29	0·91	—	Anisocytosis and poikilocytosis, occasional Jolly bodies.
5/2/26	,,	5·0	12	33	1·6	6,000	59	0	41	0	0	1·19	0·87	—	Anisocytosis and poikilocytosis.
8/2/26	,,	6·6	17	48	1·9	7,000	32	3	65	0	0	1·05	0·82	—	Anisocytosis and poikilocytosis.
10/2/26	,,	6·5	20	42	1·9	7,000	25	1	74	0	0	0·91	0·86	—	Anisocytosis and poikilocytosis.

Viscosity from 30/12/25–10/2/26, was determined at the following temperatures: 30, 31, 31, 30, 28, 26, 30, 28, 28, 30, 30, 30.
An average of the first five determinations is regarded as normal for this sheep for calculating the colour and volume colour indices.
For abbreviations, see Table 1.
For method of calculating colour indices, see Table 4.

GRAPH 3.

SHEEP No 11921.

Infected Haemonchus contortus
(20000 larvae)



is used, the above results must be multiplied by .14, in order to convert them into values of microns. All the meaurable cells in a field were measured as they were encountered. The values are fairly uniform. The smallest cell measured 4.3μ, the largest 5μ and the average was 4.6μ.

On 21.1.26, when without measuring any cells anisocytosis could with ease be diagnosed microscopically from stained smears, similar measurements were made. The values are: 28.0, 41.5, 30.5, 28.5, 27.0, 37.0, 31.0, 28.0, 40.0, 30.0, 40.0, 41.0, 33.5, 31.0, 36.0. The smallest cell measured 3.8μ, the largest 6.8μ and the average was 4.5μ. The variation in size is considerable.

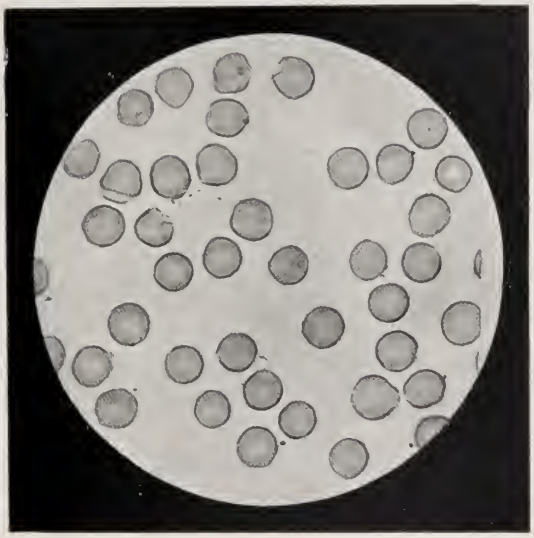
The changes in the shape of the cells (poikilocytosis) towards the end of the disease were of a most varied nature. This is well illustrated in the microphotographs Figs. 7 and 8. It is not possible to describe all the various shapes seen, but those poikilocytes more commonly encounteerd were of the shape of a pear, half-moon, bell, tennis racket, or a comma, etc. Many cells stained less intensively than others and in numerous cells are central clear areas which did not stain at all, the so-called "glasskörper" and "pessarformen," referred to by Schilling (1922). If one can draw any conclusions from the morphological study of stained smears, these cells are clearly deficient in haemoglobin, and this would explain a decrease in the haemoglobin content when no corresponding decrease occurred in the number of red cells. In other words one would expect a lower colour index thau normal, and this is actually the case (see Table 3). If however, the possibility of an error in counting be admitted, such a minus colour index will also be calculated when the counts reveal more cells than are actually present but when the haemoglobin determinations have been correct.

A decrease in the precipitate without a corresponding decrease in the number of red cells can occur when the red cells are decreased in size. On account of the extreme poikilocytosis that was present, it was useless to measure the cells, as the figures obtained would obviously have been unreliable. It can nevertheless be stated with confidence that a large number of microcytes were present.

In these smears there were not infrequently very small cells, but owing to the very marked anisocytosis and poikilocytosis it is not quite clear if these are also to be regarded as microcytes or whether they are perhaps haemoglobin fragments, the so-called schistocytes of Ehrlich, quoted by Krumbhaar (1928), or the "schizocyten" referred to by Naegeli (1912). According to this author, these cells do not occur in the bonemarrow, but only in the peripheral blood and may develop with disturbances in isotonicity, chiefly when cells of small resistance are produced in the bonemarrow. If such cells were counted as individual red cells, this would tend to produce abnormally high red counts.

Just before death there was an apparent slight improvement in the anaemia. Morphologically there was no evidence that increased regenerative changes in the haemopoietic organs was responsible for this. This was probably due to an increased concentration of the blood as a result of diarrhoea, serous atrophy of the fat, hydropericardium and disturbances in the appetite and the amount of water

SHEEP No. 11921.
FIG. 5.



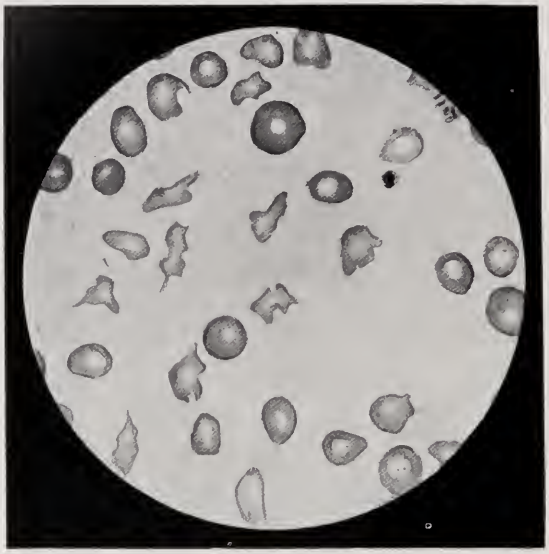
3.12.25. Normal. ×1300.

FIG. 6.

21.1.26. ×1300

Anisocytosis.

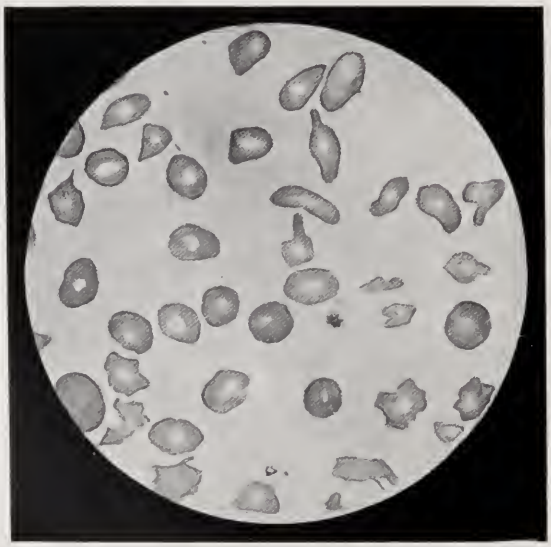
SHEEP No. 11921.
FIG. 7.



3.12.26. ×1300.

Anisocytosis and Poikilocytosis.

FIG. 8.

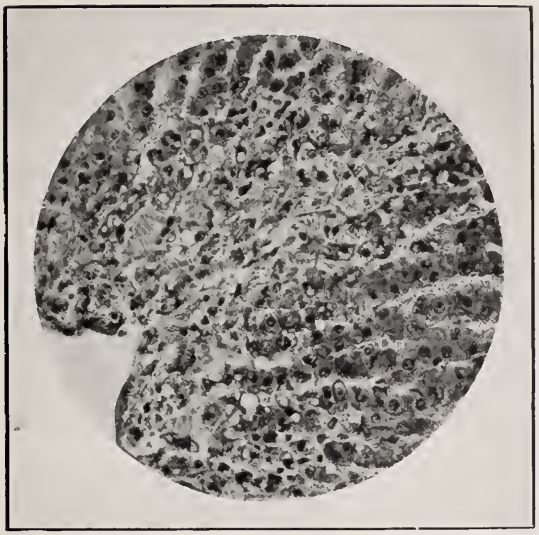


8.2.26. ×1300.

Anisocytosis and Poikilocytosis.

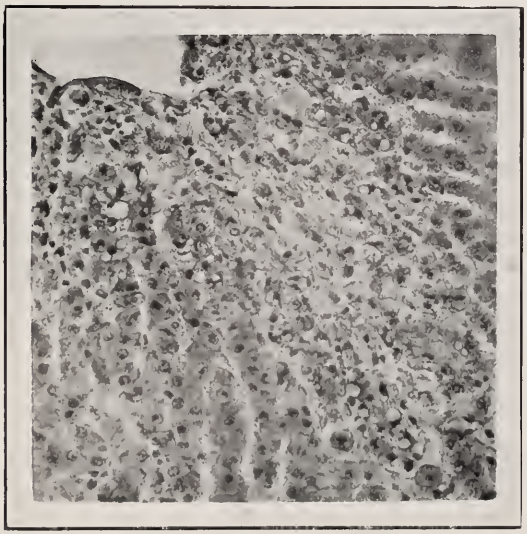
SHEEP No. 11921: SPEC. No. 5748.

FIG. 9.



Liver. ×225.
Lesions around central veins with crystals in some of the cells.

FIG. 10.



Liver. ×225.
Lesions around central veins with crystals in some of the cells.

ingested. It is a pity that absolute blood volume determinations were not made at this time. If this had been done, a decrease in the total blood volume would probably have been found to be present.

Changes in Viscosity.—There is a decided decrease in the viscosity on 21.1.26, when a very marked decrease in the number of red cells occurred, but slight changes in the number of red cells were not reflected in the viscosity curve.

Pathology of Sheep No. 11921.

Macroscopic Examination.—This sheep died on 11.2.26. The post-mortem findings were somewhat different to those recorded in previous cases, in that the condition of this ewe lamb was poor, there was marked serous atrophy of the fat and a well marked hydropericardium was present. Except for these changes, the naked eye appearances were like those previously recorded, such as pale mucous membranes, absence of icterus, clear urine, pale and watery blood, and degenerative changes in the parenchymatous organs. No parasites other than *Haemonchus contortus* were present.

Microscopic Examination.—The lesions are very similar to those described for Sheep Nos. 16027 and 11896. Cells staining a faded orange colour with Sudan III were found in all the organs except the heart. As the lesions of the liver resemble those in Sheep Nos. 16027 and 11896 combined, it is necessary to give a little more detailed description of the microscopic appearance. There are in the haemolum-eosin stained sections numerous cells with vacuoles around the central veins. These cells stain a faded orange colour with Sudan III and the crystals previously described are present in many of them. Scattered almost irregularly throughout the substance of the liver are fairly large areas, up to 113μ in cross-measurement. In these are cells containing globules staining a lemon yellow colour with Sudan III. No crystals are present in the central cells of these lesions, but cells staining the characteristic faded orange colour and containing crystals are present towards the periphery. The lesions are usually situated in the immediate vicinity of the central veins, but sometimes they occupy variable positions in the substance of the lobule. It is not quite clear whether these are to be regarded as an intermediate or a further advanced stage in the development of the lesions. In quite a number of cases the nuclei of the cells around the central veins are pycnotic and these changes are interpreted as early stages of necrobiosis.

Haematology and Pathology of Sheep Nos. 22387 *and* 22388.

Tables 4 and 5 contain the haemotological records of Sheep No. 22387 and Sheep No. 22388 respectively. Infection with *Haemonchus contortus* larvae was commenced on 5.6.29. Small doses were given until infection was completed towards the end of June, 1929. In these two sheep exact details as to the approximate number of larvae administered are not available.

TABLE 4.

SHEEP No. 22387.

Method of treatment: Infected with Haemonchus contortus *larvae from* 5.6.29.

Date.	Source.	R.C.	R.P.	Hglb. %	Serum.	W.C.	L.	M.	N.	E.	B.	V.C.I.	C.I.	V.I.	Remarks.
29/4/29	Jugular	11·3	36	84	Clear	15,700	24	5	71	0	0	0·98	0·93	0·95	Cells normal in appearance.
10/5/29	,,	9·9	34	82	,,	4,700	45	3	52	0	0	1·01	1·04	1·03	Cells normal in appearance.
5/6/29	,,	9·4	32	79	,,	5,100	56	3	41	0	0	1·03	1·05	1·02	Cells normal in appearance.
10/6/29	,,	11·0	32	84	,,	6,500	52	1	45	2	0	1·10	0·96	0·87	Occasional very large cells.
17/6/29	,,	9·8	31	75	,,	5,600	59	2	39	0	0	1·01	0·96	1·00	Cells normal in appearance.
21/6/29	,,	7·9	25	65	,,	4,500	57	1	41	1	0	1·09	1·03	0·95	Cells normal in appearance.
26/6/29	,,	5·6	19	48	,,	5,500	54	1	41	3	1	1·06	1·11	1·01	No evidence of anaemia.
1/7/29	,,	4·0	15	36	,,	5,200	59	0	40	0	1	1·00	1·13	1·12	Anisocytosis. Slight polychromasia and occasional punctate basophilia.
6/7/29	,,	2·8	15	25	,,	5,200	54	2	43	1	0	0·69	1·12	1·60	Anisocytosis, polychromasia and punctate basophilia very marked.
8/7/29	,,	2·6	19	24	,,	8,800	44	4	50	1	1	0·53	1·16	2·14	Anisocytosis, polychromasia and punctate basophilia very marked. DIED, 10/7/29.

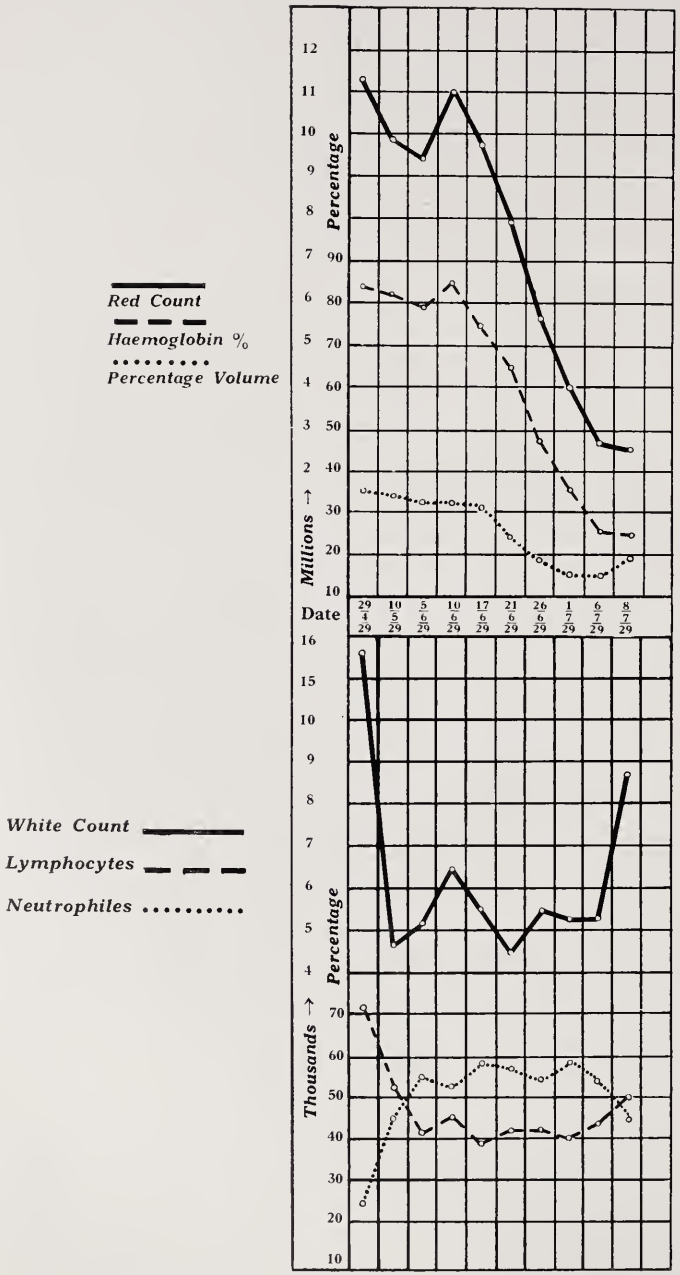
To determine the colour volume index calculate $\frac{100 \times \text{actual haemoglobin}}{\text{average normal haemoglobin (81)}} \times \frac{\text{average normal ppt. (34)}}{\text{actual ppt.} \times 100}$

To determine the colour index calculate $\frac{100 \times \text{actual haemoglobin}}{\text{average normal haemoglobin (81)}} \times \frac{\text{average normal R.C. (10·2)}}{\text{actual R.C.} \times 100}$

To determine the volume index calculate $\frac{100 \times \text{actual precipitate}}{\text{average normal precipitate (34)}} \times \frac{\text{average normal R.C. (10·2)}}{\text{actual red count} \times 100}$

Determinations on 29/4/29, 10/5/29, and 5/6/29, are regarded as normal for calculating the Volume and Colour indices.

For abbreviations, see Table I.



GRAPH 4.
SHEEP No. 22387.
Infected *Haemonchus contortus*
(20,600 larvae).

Haematology of Sheep No. 22387.

Changes in the Red Cells.—Within a week after infection there is a slight rise in the red counts and in the haemoglobin content. A corresponding increase in the percentage volume of red cells does not occur. This is possibly merely the normal variation that occurred in the number of red cells and is probably not due to increased activity on the part of the blood-forming organs. Thereafter a progressive decrease in the number of red cells and the total haemoglobin content takes place until 8.7.29, i.e. two days before death, when the red cells were 2.6 millions and the haemoglobin 24 per cent. A corresponding and progressive decrease also occurs in the percentage volume of red cells until 1.7.29; after this, instead of further decreasing, as might perhaps be expected, the precipitate remains constant until 6.7.29, and from this date until 8.7.29, i.e. in two days time, the precipitate actually increases from 15 per cent. on the two previous occasions to 19 per cent. The effects of these changes on the colour, the volume and the volume colour (saturation) indices, will be fully discussed subsequently.

Morphological Changes in the Red Cells.—Smears stained according to Pappenheim's May Grünwald-Giemsa method, were carefully examined microscopically. On 1.7.29 the first evidence of anaemia was observed. This consisted in changes such as anisocytosis, slight polychromasia and occasional punctate basophilia. Previous to this occasional very large red cells were observed, but to such no pathological significance was attributed. During the further course of the disease, these changes became progressively worse and, towards the end of the disease (6.7.29), the anisocytosis, polychromasia and punctate basophilia were very marked. In addiiton, poikilocytosis and not infrequent Jolly bodies were present, but the poikilocytosis was not of the extreme degree described for Sheep No. 11921.

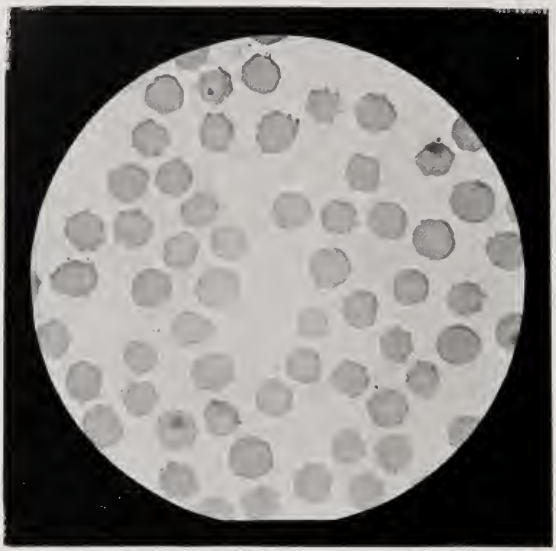
Many of the cells in the smears made during the later stages of the disease stained less intensely than normal, and in quite a large number there were central clear areas which did not stain at all. These morphological changes indicate that the cells are deficient in haemoglobin. On 8.7.29, two days before the animal died, similar changes were present, but these were, if anything, of a more severe degree.

Changes in Size of the Red Cells.—Measurements were made (20 cells) during the pre-infection period (29.4.29), and again (20 cells) when the disease was at its height (6.7.29), and a third time (40 cells) two days before the animal died (8.7.29). These results are tabulated below:—

Date.	*Smallest Cell.*	*Largest Cell.*	*Average.*
29.4.29	3.3μ	5.7μ	4.2μ
6.7.29	4.1μ	7.4μ	5.4μ
8.7.29	4.3μ	8.0μ	5.5μ

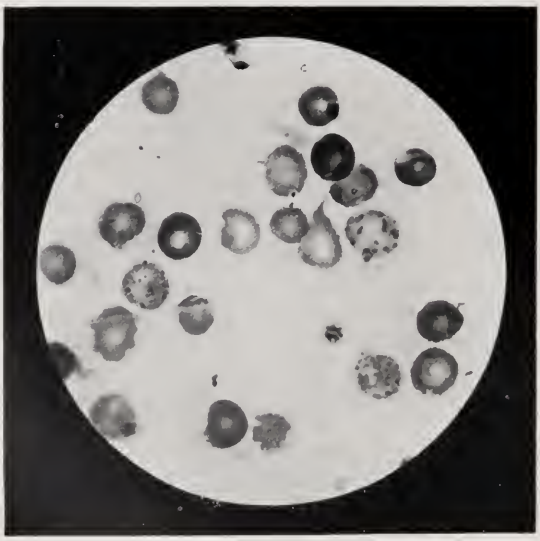
The changes in size which these measurements suggest will be much better appreciated by referring to Figs. 11, 12 and 13 which are microphotographs taken on the same day with the same magnification, of smears made from this animal during the pre-infection period (29.4.29) and later on when the anaemia was well established (6.7.29).

SHEEP No. 22387.
FATAL CASE OF HAEMONCHOSIS.
FIG. 11.



Bloodsmear made 29.4.29. ×1300.

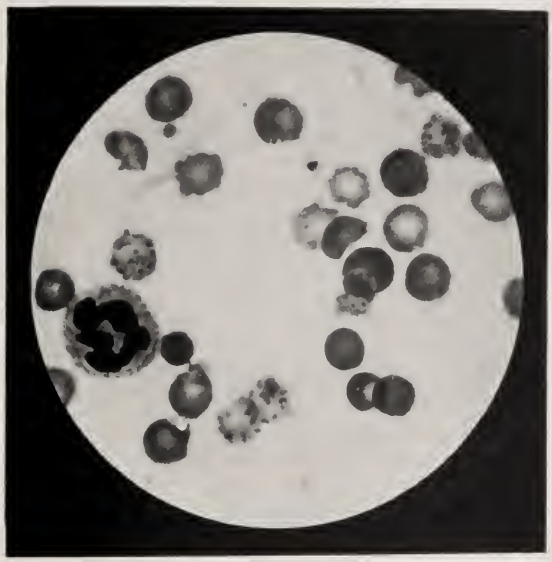
FIG. 12.



×1300.

Bloodsmear made 6.7.29, showing Anisocytosis, Punctate Basophilia and Jolly bodies.

SHEEP No. 22387.
FATAL CASE OF HAEMONCHOSIS.
FIG. 13.



×1300.

Bloodsmear made 6.7.29, showing Anisocytosis, Punctate Basophilia and Jolly bodies.

Changes in the Colour, Volume, and Volume Colour Indices.— The changes in these indices in this particular case are very interesting. In calculating the various indices in the human subject, it is usual to regard 5 million cells per c.mm. of blood as being equal to 100 per cent., the amount of haemoglobin contained in 5 million red cells per c.mm. of blood as representing 100 per cent., and the volume of packed red cells (precipitate) obtained by centrifuging a blood containing 5 million red cells per c.mm. at certain definite speeds, until such time as constant readings are obtained, as representing 100 per cent. There seems to be very little agreement as to what this last figure should actually be. Rossdale (1923) showed that anticoagulants materially affect the centrifuge readings. The values obtained by the various authors quoted by Rossdale are: Hedin (1889) 51.6, using Müller's fluid as anticoagulant and centrifuging for 5-7 minutes at a speed of 8,000 revolutions per minute; Daland (1891) 51.8, using 2.5 per cent. bichromate solution as anticoagulant and centrifuging for 3-6 minutes at a speed of 10,000 revolutions per minute, with red count of 5.07 million per c.mm.; Capps (1903) 51.5, using whole blood and centrifuging for 3 minutes at a speed of 10,000 revolutions per minute, with red counts (in males) of 5.06 million per c.mm.; Harvey (1919), average 46.5; Groen (1921), 51.4-55.1 (for males).

Rossdale (1923) himself found an average of 50, when centrifuging for 20 minutes, at a speed of 3,600 revolutions per minute. Haden (1925) used sodium oxalate as anticoagulant and centrifuged

the blood for 30 minutes at 2,500 revolutions per minute, as suggested by Hooper, Smith, Belt, and Whipple (1920). His figure is 46 for a blood containing 5 million cells. In calculating the various colour and volume indices, this author regards the average normal percentage volume of red cells at 46, which would represent 100 per cent. Whatever figure is agreed upon, as a result of examining a large number of normal individuals, as being the normal average for the human subject, that figure may differ considerably from the normal of any particular patient. When one is working with animals under experimentally controlled conditions, it is unnecessary to take as normal, the standards as laid down by various authors for particular species. It is much more accurate to lay down the normal for each individual during the pre-infection period. As there is a normal variation in the counts of each individual, one should make at least 5 determinations during the pre-infection period on different days. This was not always possible, and in some cases values obtained after infection was commenced were considered as normal. This was as a rule only done when no changes had occurred, which could be regarded as other than the normal variation. With this method, which can obviously be applied to the human subject on rare occasions only, more reliable colour and volume indices can be calculated.

When the colour index, the volume index (Capps), and the volume colour index (Neser 1921, Rossdale 1923) or the saturation index (Haden 1925) are calculated, when 5 million cells per c.mm. of blood are regarded as 100 per cent. and when the haemoglobin contained in such blood as well as the precipitate obtained from it under the conditions previously stated are regarded as 100 per cent., then in normal individuals these indices should always be 1, within admissable limits of technical error and within the limits of normal variation in an individual, that is, if normality for each individual is first of all established as advocated in this work. In this work it was not possible to investigate what variation, if any, may occur in sheep or in an individual sheep, dependent on changes in size and haemoglobin content of the red cells when technical errors have been eliminated by making in each case a number of check counts.

There are undoubtedly tremendous differences in the red counts of apparently normal sheep. Counts ranging between 10 and 14 million per c.mm. of blood are quite common, i.e. for well-grown lambs up to a year old. One would, however, expect that as the red counts become increased or decreased in normal sheep, the haemoglobin content and the percentage volume should show corresponding variations, so that the various colour and volume indices will remain unaltered, except where the cells may be increased or decreased in size and contain a proportionately increased or decreased amount of haemoglobin.

Blood containing a normal number of cells with microcytes predominating will give a relatively small precipitate and the volume index will be decreased, but on the other hand if macrocytes predominate blood with a normal number of cells will give a relatively large precipitate and the volume index will be increased. In normal individual sheep, such a deviation from the normal volume index (which is 1) may occur, if one uses the average obtained of a large

number of determinations on normal sheep as the normal for this species, but should not occur when the normality of the individual which is being examined has been determined and is being used.

The colour index is the relative ratio of the haemoglobin to the number of red cells; the volume index (Capps) is the relative ratio of the percentage volume of red cells (more conveniently termed the precipitate), to the number of red cells; the volume colour index (Neser and Rossdale) or the saturation index (Haden) is the relative ratio of the haemoglobin to the percentage volume of red cells. These can be simply calculated in the following way:—

1. Colour index =

$$\frac{100 \times actual\ haemoglobin}{average\ normal\ haemoglobin} \times \frac{average\ normal\ red\ count}{actual\ red\ count \times 100}$$

2. Volume index =

$$\frac{100 \times actual\ precipitate}{average\ normal\ precipitate} \times \frac{average\ normal\ red\ count}{actual\ red\ count \times 100}$$

3. Volume colour index or saturation index =

$$\frac{100 \times actual\ haemoglobin}{average\ normal\ haemoglobin} \times \frac{average\ normal\ precipitate}{actual\ precipitate \times 100}$$

Where the average normal red count, haemoglobin percentage and precipitate of an individual cannot be previously determined, the average normal for the species can be substituted.

The papers on the volume index by Capps (1903), Wroth (1907), and Larrabee (1911), quoted by Haden (1925), were unfortunately not available to me.

The term " volume colour " was used by Neser (1921) to express the ratio of the haemoglobin percentage to the percentage volume of red cells. Unfortunately Neser did not calculate the relative ratio, but made a direct calculation instead, simply by dividing the precipitate into the haemoglobin-percentage. His index in normal horses could, therefore, never be 1. Previous to this Herz (1893), quoted by Rossdale (1923) and Haden (1925), calculated this index in exactly the same way and called it the " specific haemoglobin content of the cell " (Specifischer Haemoglobin gehalt). In the case of a normal human blood having a haemoglobin of 100 per cent. and a red precipitate of 50, Herz's index would be 100/50.

Rossdale (1923) independently used the term volume colour index to express " the capacity of the cell volume for haemoglobin." Haden (1925) suggested for exactly the same thing the term saturation index. Both these latter workers give values which are expressed in terms of the normal. Consequently all these indices should be 1 in the case of normal adult individuals, and where they are either greater or less than 1, a deviation from the normal is indicated, particularly changes in the haemoglobin content of the cells and changes in the size of the cells.

On referring to Table IV, it will be seen that definite changes occurred in the colour index, the volume index, and the volume colour

index. Any changes occurring in these indices up to and including the 21.6.29 are probably due to technical errors, particularly in the counting of the red cells.

Changes in the Colour Index.—Towards the later stages of the disease there is a tendency to a slight increase in this index. Haden (1925) states that a colour index greater than 1.00 means only that the cells are larger than normal. Theoretically, a plus colour index would indicate either an increase in the haemoglobin content or a decrease in the number of red cells, if these are larger than normal; but in the latter case the larger and fewer cells must contain the same amount of haemoglobin as the greater number of smaller cells. There is, however, definite evidence, on morphological grounds, that the cells are to some extent deficient in haemoglobin; such deficiency would therefore tend to correct the tendency towards a plus colour index; in other words, one may actually be dealing with a pathological blood containing less, but larger cells than normal, and the colour index can remain 1.00, if there is a corresponding decrease in the amount of haemoglobin in the cells. There is very little doubt that this had occurred in this particular sheep (No. 22387). Notwithstanding, there is a slight increase in this index and one must conclude that the increase which occurred in the size of the cells was of such a degree, that it produced a plus colour index, even though the cells were deficient in haemoglobin.

Changes in the Volume Colour Index.—This index remained normal up to 1.7.29, but it was markedly decreased on 6.7.29 (5 days later), namely to .69, and a further decrease took place on 8.7.29 (2 days later) when it was .53. A decrease in the volume colour index can occur if there is a relative deficiency in the haemoglobin content of the cells, when the cells remain the same in size and number, or if the cells are decreased in number even though they may be increased in size. When, however, the larger but fewer cells are fully charged with haemoglobin, the volume colour index may remain unaltered. Theoretically, a decrease in the volume colour index can also occur if the precipitate is increased when the total haemoglobin remains unaltered. The precipitate can increase if there are more cells, which are at any rate not decreased in size, or if the cells normal in number are increased in size; but in both these cases the volume colour index will remain unchanged if the cells, whether they are normal or increased in size, are fully charged with haemoglobin. It is, therefore, obvious that when the volume colour index is decreased, the cells must be deficient in haemoglobin. As already stated, the presence of this deficiency was suggested by the morphological changes described and also by the changes in the colour index, although, admittedly, the plus colour index emphasizes rather more the increase in the size of the red cells.

Changes in the Volume Index.—Apart from slight changes, probably due to technical arrors, no changes occur in the volume index up to and including 26.6.29. On 10.6.29, however, there is a decided decrease in the volume index. This occurs within a week after infection was commenced. This was unfortunately not observed at the time and consequently the determinations could not be repeated

in order definitely to eliminate the possibility of a technical error during centrifuging. On 1.7.29 the volume index begins to increase and is decidedly increased on 6.7.29, namely to 1.6. It is even further increased 2 days later, i.e. on 8.7.29, when it is 2.14. This indicates very definitely that a marked increase in the size of the red cells has taken place. This was already suggested on general morphological grounds, and also by the measurements that were made, e.g., the average normal size of the red cells was 4.2μ during the pre-infection period, but towards the end of the disease (on 6.7.29 and 8.7.29) the average diameter of the red cells was 5.4μ and 5.5μ respectively. Slight differences in size, especially when uniform, may be difficult or impossible to appreciate microscopically, even by actually measuring the cells, but must nevertheless cause a well marked increase in the precipitate, with a consequent plus volume index. This is commonly encountered in early cases of pernicious anaemia in the human subject.

From a purely haemotological point of view, the outstanding features of this particular case are: marked morphological evidence of anaemia, e.g., anisocytosis, poikilocytosis, polychromasia, "glasskörper" and "pessarformen," punctate basophilia, and Jolly bodies. There is a slight increase in the colour index, a marked decrease in the volume colour or saturation index, and a marked increase in the volume index. These changes are interpreted as indicating that there is a relative deficiency in haemoglobin in the cells and that there is a marked increase in the size of the cells. Some of the macrocytes can appropriately be classified as megalocytes, but no megaloblasts, so characteristic of pernicious anaemia in the human subject, were recognized. Except for the absence of these last-named cells, the blood changes recorded in this particular animal, affected with a pure haemonchosis, correspond very closely with those described for pernicious anaemia in the human subject. Haden's (1925) average for 50 cases of pernicious anaemia is: Volume index 1.41, colour index 1.29, saturation index .92. His highest volume index is 1.77, with a saturation index of .53. This corresponds very closely with the indices of Sheep No. 22387, but the colour index in pernicious anaemia seem in general to be slightly higher.

Changes in the Leucocytes.—There is a remarkable leucocytosis with a neutrophilia on 29.4.29. This occurs during the pre-infection period and no explanation of the cause of this can be given. As occurs in other cases, there is a leucocytosis with a neutrophilia towards the end of the disease.

Pathology of Sheep No. 22387.

The animal which was a young sheep and had not yet shed any of its temporary or milk teeth, died on 10.7.29, when a post-mortem examination was made. The same general changes as described for previous cases were recorded. There was in addition a very interesting lesion which involved mainly the apex of the heart. There was thrombosis or embolism of one of the branches of a coronary artery, resulting in infarction of the myocardium in the region of the apex. A similar lesion was encountered in some of the other sheep with haemonchosis and this will be referred to again later on.

This animal must have died during the early part of the evening of 9.7.29, and was available for post-mortem examination only on the morning of 10.7.29, when a certain amount of autolysis and decomposition had unfortunately already occurred, particularly in the liver. In these circumstances it is not possible to make a definite statement in regard to specific lesions, but the outstanding features of the macroscopic and microscopic examinations are: absence of demonstrable iron pigment from all the organs, including even the spleen, which was markedly atrophied, being of small size and having a very much wrinkled capsule. There was general paleness of the visible and other mucous membranes, and of the tissues in general. There was no icterus. Microscopically the bonemarrow was found to contain the usual immature forms of granulocytes and erythrocytes, such as myeloblasts and myelocytes, etc. The presence of these cells was interpreted as indicating an actively functioning bonemarrow. In the fatty bonemarrow were seen scattered groups or islands of cells, many of which were neutrophile and cosinophile myelocytes, while others were erythroblasts and normoblasts. This was interpreted as a compensatory myeloid hyperplasia involving the fatty bonemarrow. No parasites other than wireworms were found to be present.

Haematology of Sheep No. 22388.

Changes in the Red Cells.—On studying Table 5, with its corresponding graph, it will be observed that there is during the pre-infection period a peculiar decrease in the red counts and the absolute haemoglobin content of the cells, as well as in the precipitate. The cause of this is not definitely known, but may possibly be associated with the artificial conditions under which these animals were placed, when they were drafted into this experiment. As previously explained, these animals were obtained from a Karroo farm, where the conditions for parasitic life are generally unfavourable. On arrival at the Onderstepoort Laboratory, they were immediately placed in a loose box measuring 6.25 by 3 metres. This box was subdivided by 5 partitions into oblong compartments in each of which were placed 2 to 3 sheep. The sheep had a liberal supply of good food and abundant water, but obviously exercise was restricted to an absolute minimum. Neser (1923) showed that a remarkable increase in the number and in the percentage volume of red cells takes place with exercise or work in horses. No observations of this nature have so far been attempted on sheep under experimentally controlled conditions, but there is just a possibility that this may be the explanation of the marked decrease in the number of red cells that occurred in this sheep. Arguing by analogy, this should not be the case, as Neser has shown that in horses the reverse process, that is, decrease of the red cells with idleness and restricted exercise is slow. This may, however, not be the case with sheep. It is noteworthy that a similar decrease in the red cells of Sheep No. 22387 (see Table 4 and Graph 4) was recorded. This animal was bought from the same Karroo farm, arrived here at the same time and was further treated in exactly the same way as was Sheep No. 22388. This suggests that exercise will have the same interesting effects on the red cells of sheep as was shown to be the case in horses by Neser and in cattle by Canham (1930).

TABLE 5.

SHEEP No. 22388.

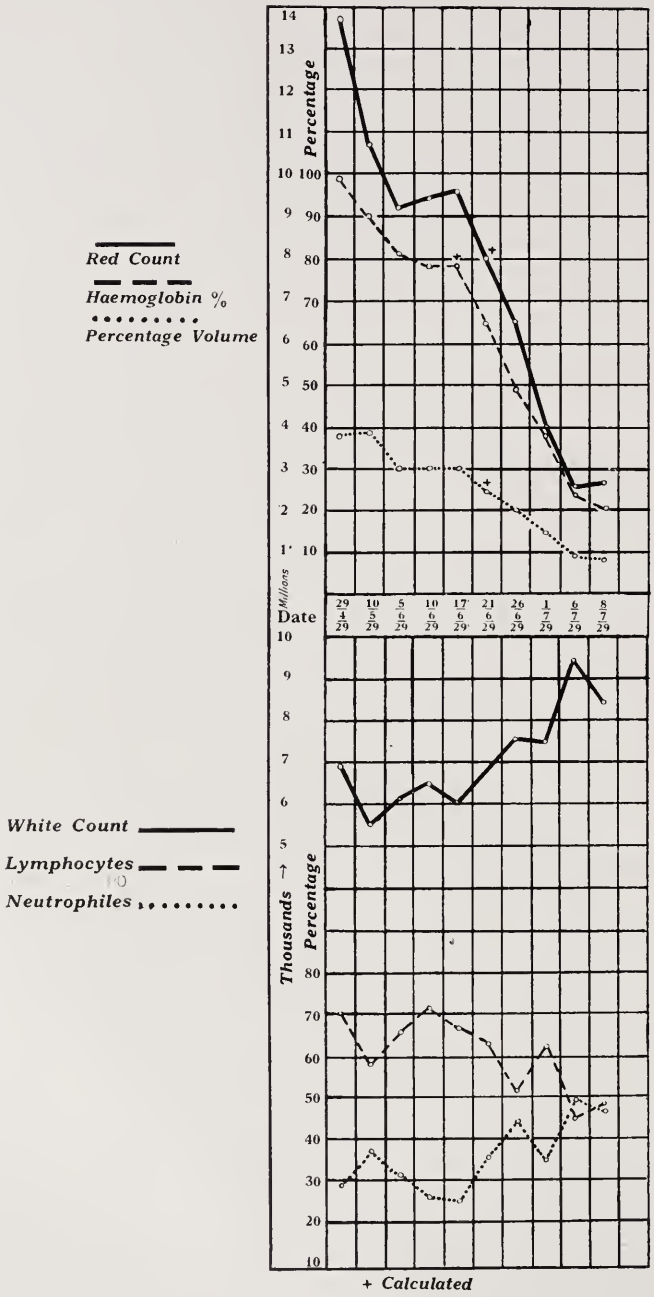
Method of treatment: Infected with Haemonchus contortus *larvae from* 5.6.29.

Date.	Source.	R.C.	R.P.	Hglb. %	Serum.	W.C.	L.	M.	N.	E.	B.	V.C.I.	C.I.	V.I.	Remarks.
29/4/29	Jugular	13·8	38	99	Clear	6,900	70	0	29	0	1	0·92	0·85	0·86	Cells normal.
10/5/29	,,	10·7	39	90	,,	5,500	58	3	37	1	1	0·88	1·00	1·14	Cells normal.
5/6/29	,,	9·2	30	81	,,	6,100	66	3	31	0	0	1·03	1·05	1·02	Occasional fairly large cells.
10/6/29	,,	9·4	30	78	,,	6,500	71	2	26	1	0	0·99	0·99	1·00	Cells normal.
17/6/29	,,	9·6	30	78	,,	6,000	67	3	25	4	1	0·99	0·92	0·98	Cells normal.
21/6/29	,,	8·0	24	65	,,	—	63	1	35	1	0	1·03	0·97	0·94	Cells normal.
26/6/29	,,	6·6	20	49	,,	7,600	51	3	44	2	0	0·93	0·88	0·95	Anisocytosis and punctate basophilia.
1/7/29	,,	4·0	14	39	,,	7,500	62	1	34	3	0	1·06	1·16	1·10	Anisocytosis and poikilocytosis.
6/7/29	,,	2·6	9	23	,,	9,400	45	2	49	3	1	0·97	1·05	1·08	Anisocytosis, poikilocytosis and punctate basophilia.
8/7/29	,,	2·7	8	20	,,	8,400	48	5	47	0	0	0·95	0·88	0·93	Anisocytosis, poikilocytosis and punctate basophilia. DIED 10/7/29.

The average of figures obtained on 10/5/29, 5/6/29, and 10/6/29, is regarded as normal for calculating the colour and volume indices. The figures obtained on 29/4/29 and 10/5/29, were rejected as being probably inaccurate.

For abbreviations, see Table 1.

For method of calculating colour indices, see Table 4.



GRAPH 5.
SHEEP No. 22388.
Infected *Haemonchus contortus*
(20,600 larvae).

If restricted exercise was responsible for the decrease in the number of red cells and the corresponding decrease in the haemoglobin content and the percentage volume of red cells, it is difficult to say whether the blood reached its normal level for conditions of complete inactivity on 5.6.29 (see Graph 5) or whether the downward trend of the curve was checked as from 5.6.29 when infection was commenced, as a direct result of the action of *Haemonchus* larvae in producing an initial stimulating effect on the blood-forming organs.

From 17.6.29 until 6.7.29, there is a progressive decrease in the number of red cells, together with a decrease in the amount of haemoglobin and in the pricipitate. The rate of decrease in the red cells varies from 1.5 to 2.5 millions per week. The course of the disease in this sheep as well as in Sheep No. 22387 can be regarded as acute or even peracute, as it is not often that sheep will die under artificial conditions of infection, from a pure *Haemonchus* infestation within 5 weeks after infection was commenced.

On 8.7.29 the red count and the precipitate remain practically the same, but there is a decrease in the haemoglobin content as compared with the figures obtained on 6.7.29.

Changes in the colour, volume, and volume colour indices.—The figures obtained during the pre-infection period, i.e. on 29.4.29 and 10.5.29, were regarded as abnormal, consequently they were rejected when calculating the average normal red count, haemoglobin percentage, and precipitate. The average of the figures obtained on 5.6.29 (blood drawn on this date can also be regarded as having been collected during the pre-infection period) 10.6.29, and 17.6.29, was regarded as the average normal for this sheep.

Changes in the colour index.—No marked changes occur in this index. In the early stages of the anaemia there is a tendency towards a decrease in this index, which is calculated as .88 on 26.6.29. Within a week after this there is a plus colour index and towards the end of the disease the index again decreases. A decrease in the colour index is due either to a deficiency in haemoglobin, or, if the cells are fully charged with haemoglobin, to a decrease in the size of the red cells, which means that there must be a relatively greater number of these smaller cells in order to supply the normal amount of haemoglobin, that is, assuming that supersaturation of the cells with haemoglobin did not occur. It is difficult to measure directly a decrease in the haemoglobin content of the cells, but morphological evidence of anaemia, such as anisocytosis and punctate basophilia, was already present on 26.6.29. The other factor, namely the decrease in the size of the red cells, can be determined by direct measurement. Fifteen cells were measured on two occasions (10.5.29 and 5.6.29), when the animal's blood can be regarded as normal, 15 cells were measured during the early stages of the anaemia (26.6.29), and 40 cells were measured when the animal had developed a severe degree of anaemia. These results are tabulated below:—

Date.	*Smallest cell.*	*Largest cell.*	*Average.*
10.5.29	3.8μ	4.6μ	4.2μ
5.6.29	4.1μ	4.8μ	4.5μ
26.6.29	3.5μ	5.1μ	4.5μ
1.7.29	4.1μ	5.7μ	5.0μ

It must again be emphasized that too few cells were measured to permit of the averages obtained being regarded as even approximately true for the smears of that particular date, but the measurements suggest that no decrease in size has occurred. According to these measurements no changes in the size of the red cells occur up to and including 26.6.29. The decreased colour index that is present on this date would, therefore, appear to be due to a relative deficiency in haemoglobin. On 1.7.29 there is definite evidence that the cells have increased in size and this is responsible for the plus colour index on that date. The more or less normal colour index on 6.7.29 is probably due to a relatively greater deficiency in haemoglobin, in spite of the increase in size which is probably still present, as the volume indices on 1.7.29 and 6.7.29 are practically the same. Owing to the severe degree of poikilocytosis, it was useless measuring the cells on the latter date, as too great a selection would have had to be practised in picking out the measurable cells. On 8.7.29 the colour index is again decreased and is practically the same as on 26.6.29.

Changes in the volume index.—One is probably not justified in interpreting the slight decrease in the volume index on 17.6.29, 21.6.29, and 26.6.29 as being of pathological significance. A plus volume index is present on 1.7.29 and 6.7.29, and this indicates that an increase in the size of the red cells has occurred on these dates. The measurements previously quoted tend to confirm this.

Changes in the volume colour index.—No marked changes occur in this index. There is a slight decrease on 26.6.29, when the colour index also is decreased and this tends to confirm the conclusion that a relative deficiency in haemoglobin was present on this date. Towards the end of the disease there is a slight tendency towards a decrease in this index.

Changes in the leucocytes.—During the pre-infection and post-infection periods, there is a good deal of fluctuation in the total white counts, but there is a tendency throughout the entire course of the disease towards a gradual rise in the total number of leucocytes. On referring to the differential counts, it will be seen that this increase in the number of leucocytes is due to an increase in the number of neutrophiles, and this commences at the same time, as the, so to speak, pathological decrease in the number of red cells occurs. This continuous increase in the number of neutrophiles is interrupted on 1.7.29, when these cells decrease from 44 per cent. to 34 per cent. It was not possible to determine whether this has any special pathological significance. On the other hand, the neutrophilia indicates a healthy activity on the part of the blood-forming organs, undoubtedly stimulated thereto as the result of the loss of red cells.

Pathology of Sheep No. 22388.

The animal died during the night of 9.7.29 and was available for post-mortem examination on 10.7.29. It is unnecesary to give a detailed description of all the organs, and reference will only be made to outstanding features of this case: There was a very marked paleness of the visible and other mucous membranes, as well as of all the tissues throughout the body. The blood was watery and stained

badly. The animal was in poor condition. There was very marked atrophy of the spleen, and hydropericardium, oedema of the lungs, absence of icterus and haemoglobinuria, and what were interpreted macroscopically as degenerative changes in the parenchymatous organs. Numerous wireworms were present in the abomasum, the mucous membrane of which contained numerous dark red spots. The ingesta of the abomasum and of portions of the small intestine were of a chocolate colour and strongly positive for haemoglobin when tested chemically with guiaconic acid. No other parasites besides wireworms were found to be present.

Microscopic examination.—Liver: The cells around the central veins stain less intensely and contain a fair amount of true fat in the form of small droplets, otherwise no changes are present and the crystals described in other cases cannot be identified. Kidneys: Slight fatty changes are the only lesions identified. Bonemarrow: In the case of both the red and the yellow marrow the picture is the same as described for Sheep No. 22387.

(B) Recovery Cases.

Quite a number of cases were encountered in which the infective larvae that were administered developed to maturity and subsequently passed eggs (found in the faeces), without producing any noticeable effects on the hosts. The nature of this immunity or resistance was not studied.

A number of other sheep developed varying degrees of anaemia and then recovered, with a gradual improvement of the anaemia. These can conveniently be referred to as recovery cases. Of quite a number of such cases encountered during the course of this investigation, the haematological details of three will be presented. These are Sheep No. 15699 (Table 6 and Graph 6), Sheep No. 15680 (Table 7 and Graph 7) and Sheep No. 11828 (Table 8 and Graph 8).

Sheep No. 15699 was infected with 3,000 larvae, which were given in one dose on 17.11.26. As will be seen on referring to Table 6 and Graph 6, this animal was not examined haematologically during the pre-infection period. The first counts, made 10 days after infection, are extremely high even for a young sheep. It cannot be stated positively that, on the day (27.11.26) when these counts were made, conditions such as polyuria, diarrhoea, lack of water, etc., which produce overconcentration of the blood, were absent. As a reult of the infestation, the red counts decreased rapidly until 22.12.26. This was accompanied by a corresponding decrease in the haemoglobin and the precipitate. After this date there is a gradual improvement in the anaemia. The helminthological records of this sheep are unfortunately not available, so that it is not possible to state definitely whether the animal continued to pass eggs in its faeces after 22.12.26 when recovery commenced. If one can draw any conclusions from the study of Graph 6, it would seem that the animal had not entirely lost its infection and that during the period from 7.1.27 to 11.1.27, the parasites again produced a decrease in the number of red cells, with a corresponding decrease in the haemoglobin and the percentage volume of red cells. Thereafter the blood again improved until 10.2.27, when a second setback occurred. After that the animal made an uneventful recovery.

TABLE 6.

SHEEP No. 15699.

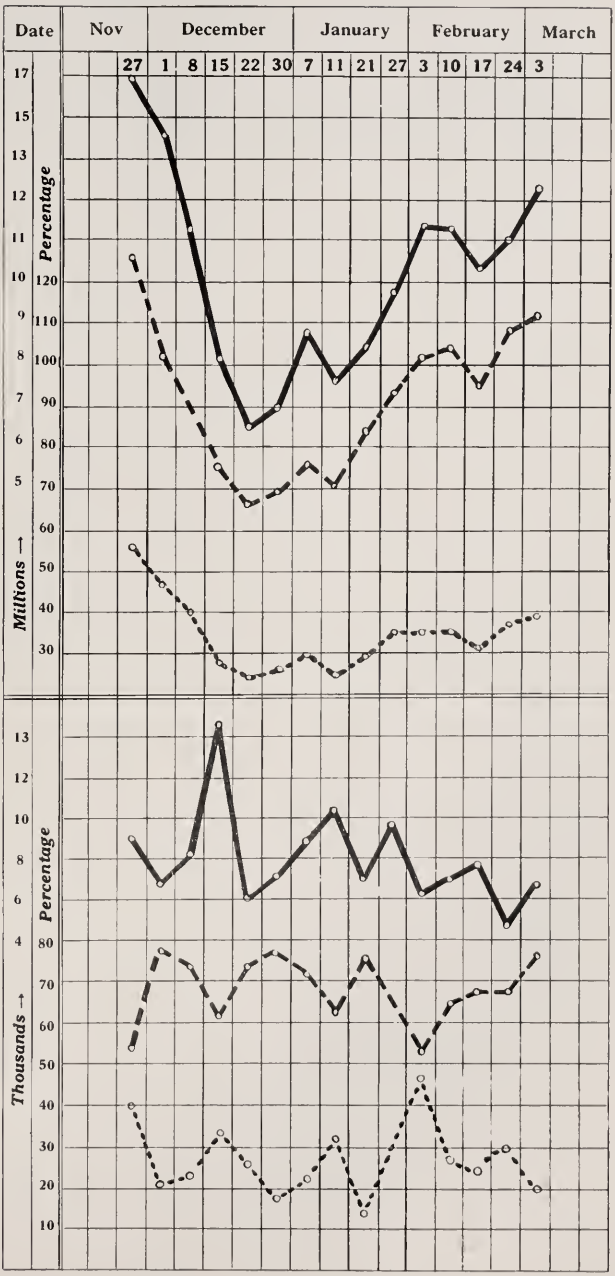
RECOVERY CASE.

Method of treatment: Infected with Haemonchus contortus, 3,000 *larvae, on* 17.11.26.

Date.	Source.	R.C.	R.P.	Hglb. %	W.C.	L.	M.	N.	E.	B.	V.C.I.	C.I.	V.I.	Remarks.
27/11/26	Jugular	17·1	56	126	9,000	54	1	40	3	2	–	—	—	No morphological changes in red cells.
1/12/26	,,	14·2	47	102	6,700	78	0	21	1	0	—	—	0·76	—
8/12/26	,,	11·3	40	86	8,200	74	0	23	2	1	—	—	0·75	—
15/12/26	,,	8·2	28	76	13,300	62	2	34	2	0	—	—	0·95	—
22/12/26	,,	6·6	24	67	6,000	74	0	26	0	0	—	—	0·98	—
30/12/26	,,	7·0	26	69	7,100	77	4	18	0	1	—	—	0·93	—
7/12/27	,,	8·8	30	76	8,700	72	3	22	2	1	—	—	0·88	Serum pale yellow and cloudy. White layer on ppt.
11/1/27	,,	7·7	24	71	10,200	63	1	32	4	0	—	—	1·00	—
21/1/27	,,	8·5	29	84	7,000	77	0	14	7	2	—	—	1·00	—
27/1/27	,,	9·8	35	93	9,800	Smear bad, unable to count.					—	—	0·93	—
3/2/27	,,	11·5	35	102	6,100	52	0	47	1	0	—	—	1·00	—
10/2/27	,,	11·4	35	104	6,900	65	1	28	1	5	—	—	1·00	—
17/2/27	,,	10·4	31	96	7,800	68	1	26	4	2	—	—	1·08	—
24/2/27	,,	11·0	37	108	4,700	68	0	30	1	1	—	—	1·02	—
3/3/27	,,	12·4	39	111	6,600	77	0	20	3	0	—	—	0·99	—

In calculating the volume index the average of the last 6 counts is regarded as normal.
For abbreviations, see Table 1.
For method of calculating colour indices, see Table 4.

GRAPH 6.
SHEEP No. 15699.
RECOVERY CASE.



On studying Table 7 with its Graph 7, and Table 8 with its Graph 8, which refer to Sheep Nos. 15680 and 11828 respectively, it will be found that practically similar results were obtained when 10,000 larvae were administered to Sheep No. 15680 (Graph 7) in one dose, and 20,600 larvae were administered to Sheep No. 11828. In the latter sheep infection was commenced on 16.11.25. 200 larvae were given every second day until 600 larvae had been given, and thereafter 2,000 larvae were given every second, third or fourth day, until infection was completed on 23.12.25.

TABLE 7.

SHEEP No. 15680.

RECOVERY CASE.

Method of treatment: Infected with Haemonchus contortus, 10,000 *larvae, from* 17.11.26.

Date.	Source.	R.C.	R.P.	Hglb. %	W.C.	L.	M.	N.	E.	B.
16/11/26...	Jugular	13·3	37	99	7,200	87	0	5	8	0
24/11/26...	,,	14·1	40	104	10,100	80	1	15	4	0
1/12/26....	,,	11·6	35	93	8,700	80	2	17	0	1
8/12/26....	,,	8·0	25	69	8,600	83	1	16	0	0
11/12/26.	,,	6·3	19	54	8,400	81	3	16	0	0
15/12/26...	,,	6·6	21	59	7,300	58	1	40	0	1
22/12/26...	,,	5·7	20	57	7,700	81	3	15	1	0
30/12/26...	,,	5·1	19	56	14,000	53	0	47	0	0
7/1/27.....	,,	6·1	17	52	6,300	75	2	23	0	0
11/1/27....	,,	6·9	19	59	7,700	62	4	33	0	1
21/1/27....	,,	6·8	24	65	9,700	69	1	30	0	0
27/1/27....	,,	8·2	25	69	7,600	75	3	20	2	0
3/2/27.....	,,	7·9	25	74	3,700	82	0	16	0	2
10/2/27....	,,	7·7	26	76	6,300	81	0	19	0	0
17/2/27....	,,	9·3	28	80	5,500	74	2	18	6	0
3/3/27...	,,	8·1	24	71	6,600	83	3	11	3	0

For abbreviations, see Table 1.
For method of calculating colour indices, see Table 4.

GRAPH 7.

SHEEP No. 15680.

Recovery Case.

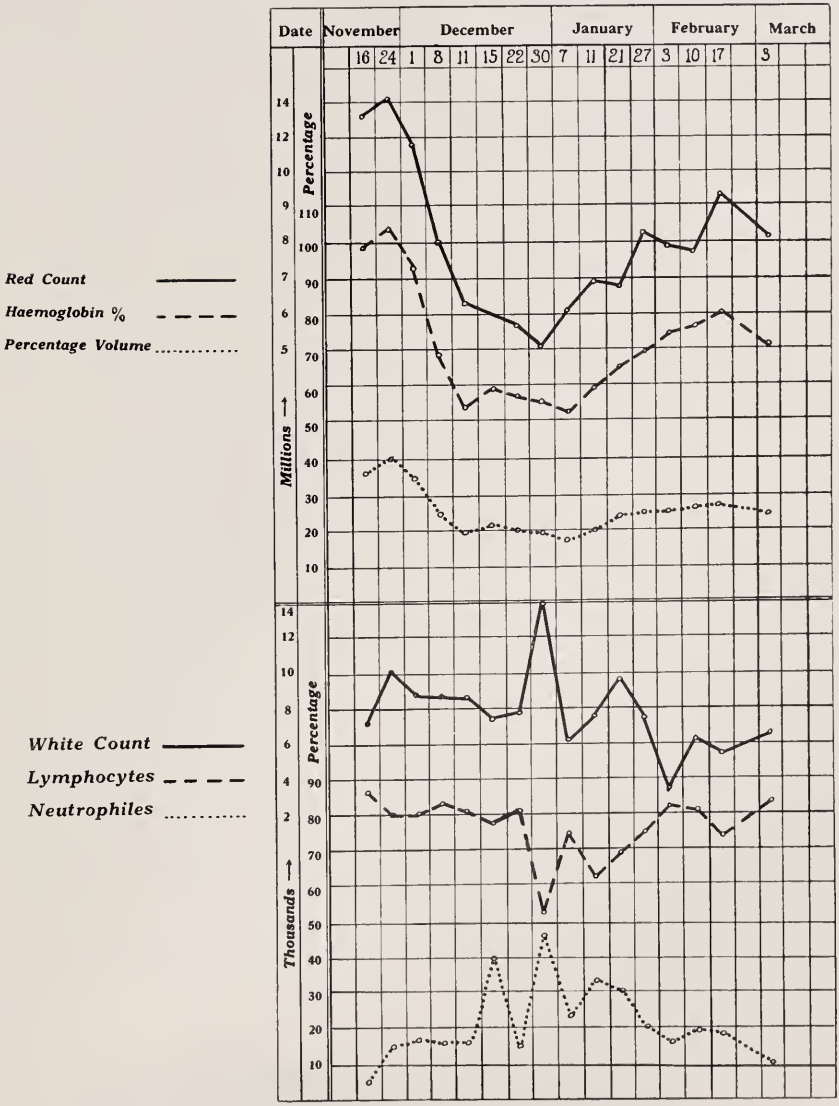


TABLE 8.

SHEEP No. 11828.

RECOVERY CASE.

Method of treatment: Infected with Haemonchus contortus, 20,600 *larvae.*

Date.	Source.	R.C.	R.P.	Hglb. %	Viscosity.	W.C.	L.	M.	N.	E.	B.	Remarks.
3/11/25	Jugular	14·6	35	91	—	10,000	59	2	35	4	0	1st dose.
5/11/25	,,	13·0	33	87	—	11,000	62	4	29	5	0	(16/11) 200, (18/11) 200, (20/11)
9/11/25	,,	15·7	34	90	—	8,800	44	5	50	1	0	300, (2/12) 2,000, (4/12) 2,000,
11/11/25	,,	14·3	33	87	—	9,000	50	3	41	6	0	(7/12) 2,000, (9/12) 2,000, (11/12)
19/11/25	,,	14·9*	39	96	—	8,600	58	2	31	9	0	2,000 (14/12) 2,000, (17/12)
24/11/25	,,	15·9	43	108	—	8,200	61	2	34	2	1	2,000 (19/12) 2,000, (21/12) 2,000
1/12/25	,,	17·2	44	114	—	12,700	21	3	72	4	0	(23/12) 2,000 = 20,600 larvae.
9/12/25	,,	15·7	39	107	—	8,700	56	2	38	4	0	—
15/12/25	,,	16·9	41	104	—	11,700	47	2	42	9	0	—
22/12/25	,,	17·4	42	107	—	11,200	44	3	51	2	0	—
28/12/25	,,	16·3	39	100	—	8,800	69	1	25	3	2	—
6/1/26	,,	11·6	34	81	3·2	7,800	60	2	29	8	1	—
12/1/26	,,	12·0	34	79	3·4	7,800	50	0	46	2	2	—
19/1/26	,,	11·5	33	73	3·5	7,000	74	3	17	6	0	—
26/1/26	,,	9·3	25	61	2·7	6,700	60	1	38	1	0	—
2/2/26	,,	9·1	20	56	2·0	11,400	60	3	35	2	0	—
8/2/26	,,	9·3	23	60	2·3	9,200	60	1	35	2	2	—
15/2/26	,,	12·8	26	75	3·2	23,000	21	2	75	2	0	—
22/2/26	,,	10·7	25	67	3·2	9,500	20	3	76	0	1	—
1/3/26	,,	12·4	33	79	3·2	13,400	44	3	50	2	1	—
8/3/26	,,	13·1	34	80	3·3	9,300	—	—	—	—	—	—
16/3/26	,,	13·5	38	87	3·2	10,100	46	2	50	2	0	—
23/3/26	,,	14·4	39	92	3·6	11,800	51	4	42	3	0	—
29/3/26	,,	16·3	41	101	3·7	8,500	50	1	36	13	0	—
6/4/26	,,	13·4	36	90	4·1	9,700	35	1	64	0	0	—
12/4/26	,,	13·8	36	89	3·9	11,500	49	3	43	5	0	—
26/4/26	,,	15·9	39	99	4·1	7,300	56	2	41	0	1	—
3/5/26	,,	14·0	36	98	4·3	8,700	50	1	47	2	0	—
10/5/26	,,	15·5	37	96	3·9	11,000	52	0	45	3	0	—
17/5/26	,,	16·1	39	108	4·8	10,600	38	2	56	4	0	—
2/6/26	,,	14·3	33	92	3·9	8,900	54	1	41	4	0	—
23/6/26	,,	—	—	95	4·7	—	54	0	46	0	0	—
29/6/26	,,	13·5	40	91	4·0	11,400	40	1	59	0	0	—
26/7/26	,,	15·4	40	92	4·11	11,700	51	1	43	5	0	—
18/9/26	,,	13·8	44	117	—	18,200	12	2	85	0	1	—

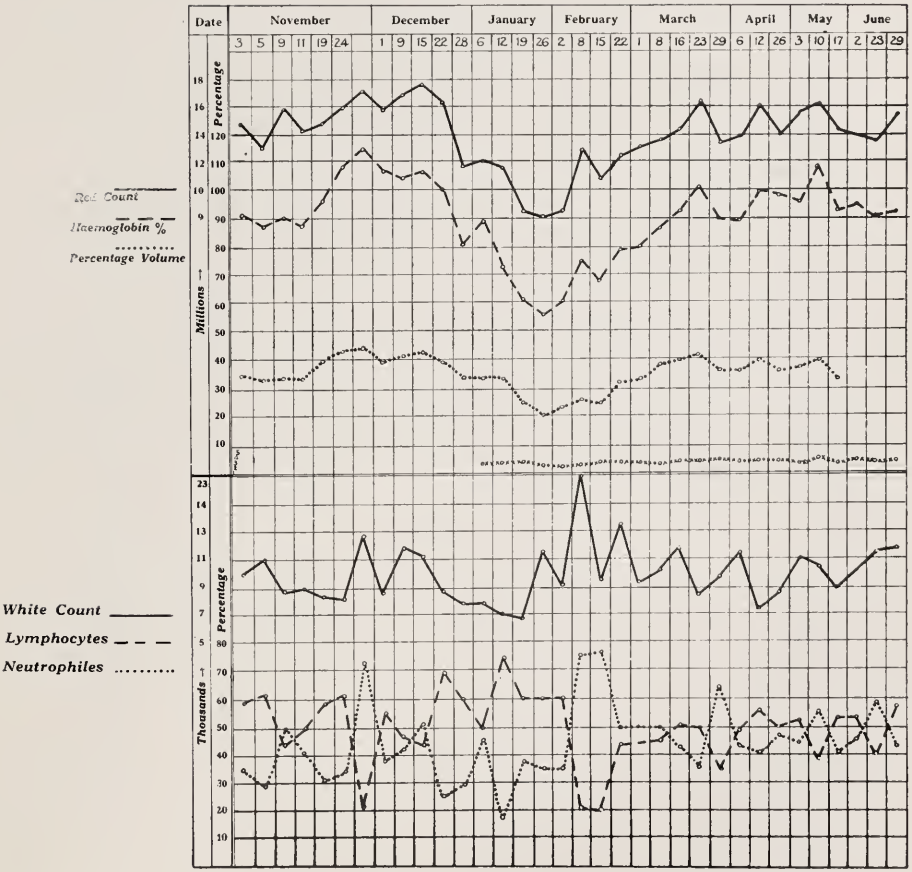
For abbreviations, see Table 1.

For method of calculating colour indices, see Table 4.

GRAPH 8.

SHEEP No. 11828.

Recovery Case.



In all these three cases it is not possible to state whether the animals completely got rid of the infection, or whether the infection remained and they developed an immunity as a result of which they were able to resist or completely to neutralize the effects of the parasites.

In view of the self cures reported by Stoll (1929), in the case of two sheep, it is necessary to refer to a further recovery case that was encountered during the course of this investigation. This Sheep No. 11814, haematological records of which are presented in Table 11 and Graph 11, was at first used in a nodular worm experiment. The animal became at the same time accidentally, but naturally, infected with wireworms. This was diagnosed by means of faeces cultures. As a result of the wireworm infection, the animal developed a fairly severe degree of anaemia, the red counts decreasing from a normal of 12-14 million to 5.5 million red cells per c.mm. of blood (16.3.26). After this the anaemia gradually improved. When this was observed, the animal was reinfected with large doses of *Haemonchus contortus* larvae, but completely resisted the infection, and when the blood was finally examined, months later (27.11.26), the animal was, from a haematological point of view, quite normal. In this work no attempt was made to study the question of immunity in, or resistance to haemonchosis in sheep. This question of acquired immunity with metazoan and other parasites is becoming increasingly important, as was shown by Taliaferro (1929) and other authors quoted by him. On referring to the differential counts (Table 11 and Graph 11), it will be observed that an undoubted eosinophilia occurs during the time the animal is recovering from the anaemia. It is noteworthy that de Kock and Quinlan (1926) also recorded an eosinophilia in splenectomized sheep at the time recovery from the anaemia due to Anaplasmosis was taking place.

(C) Controls.

As previously stated, quite a number of uninfected control sheep were always kept separate from the infected sheep, but otherwise under identical conditions of housing, feeding and watering. These control animals remained healthy and never developed any clinical symptoms. The haematological records of two of these sheep are given below.

Table 9 and Graph 9 refer to Sheep No. 11929. This is a young sheep free from worms. There is what is interpreted as a normal variation and fluctuation in the red counts, the haemoglobin and the percentage voume of red cells. In isolated cases it will be oberved that the haemoglobin and precipitate curves do not follow the red count curves. This is probably due to errors in the red counts. There is perhaps a slight downward trend of the curves in Graph 9. This is possibly due to the fact that in very young sheep the red cells decrease in number as such sheep grow older.

TABLE 9.

SHEEP No. 11929.

CONTROL.

Date.	Source.	R.C.	R.P.	Hglb. %	Viscosity.	W.C.	L.	M.	N.	E.	B.
4/11/25	Jugular	12·4	39	81	—	8,000	49	1	50	0	0
6/11/25	,,	11·6	32	73	—	9,500	51	5	43	1	0
10/11/25	,,	11·4	34	78	—	6,100	55	1	44	0	0
13/11/25	,,	14·8	42	87	—	6,400	76	2	22	0	0
20/11/25	,,	11·4	36	83	—	8,800	55	3	42	0	0
26/11/25	,,	12·1	34	78	—	5,500	74	4	21	1	0
3/12/25	,,	12·1	35	81	—	7,800	53	2	45	0	0
11/12/25	,,	10·6	34	75	—	5,600	58	5	36	1	0
18/12/25	,,	11·1	32	69	—	7,400	51	2	46	1	0
24/12/25	,,	12·0	37	81	—	9,300	51	2	47	0	0
30/12/25	,,	13·6	43	91	3·5	6,800	68	3	27	2	0
8/1/26	,,	—	40	79	3·2	7,100	61	0	39	0	0
14/1/26	,,	10·0	30	72	3·1	4,800	64	1	32	0	3
21/1/26	,,	10·3	31	76	4·0	4,200	66	1	33	0	0
23/1/26	,,	13·3	38	87	4·0	5,900	67	0	33	0	0
28/1/26	,,	11·0	30	76	3·0	5,500	60	5	32	3	0
3/2/26	,,	11·0	31	76	2·9	4,700	62	0	38	0	0
10/2/26	,,	11·7	35	84	2·5	5,900	38	0	60	1	1
17/2/26	,,	12·0	36	87	3·3	6,100	53	0	47	0	0
23/2/26	,,	11·9	31	74	3·2	6,300	58	1	41	0	0
2/3/26	,,	10·3	29	70	2·8	6,100	54	2	41	3	0
9/3/26	,,	11·1	32	75	—	4,300	50	0	50	0	0
19/3/26	,,	11·3	29	72	3·1	5,100	62	1	34	2	1
26/3/26	,,	9·8	25	62	2·6	4,700	72	3	24	0	1
30/3/26	,,	11·7	32	77	3·0	3,400	55	3	42	0	0
9/4/26	,,	10·5	28	70	2·8	5,600	47	1	52	0	0
16/4/26	,,	9·8	25	64	2·7	5,200	64	0	34	2	0
30/4/26	,,	11·8	33	81	3·1	4,500	68	3	29	0	0
5/5/26	,,	12·0	33	78	3·0	6,100	53	3	44	0	0
14/5/26	,,	10·6	—	73	2·8	6,200	50	0	48	0	2
21/5/26	,,	9·4	28	71	3·0	4,900	67	2	31	0	0
4/6/26	,,	12·2	33	84	3·0	3,000	59	1	38	0	2

For abbreviations, see Table 1.
For method of calculating colour indices, see Table 4.

GRAPH 9.

SHEEP No. 11929.

CONTROL YOUNG SHEEP.

Table 10.

CONTROL SHEEP No. 13601.

Date.	Source.	R.C.	R.P.	Hglb. %	Visco-sity.	W.C.	L.	M.	N.	E.	B.
6/1/26	Jugular	8·6	31	79	3·0	8,100	81	2	16	1	0
7/1/26	,,	8·6	29	81	3·2	7,400	70	2	26	2	0
8/1/26	,,	9·3	33	78	3·0	6,400	73	5	18	2	2
11/1/26	,,	9·0	34	81	—	6,400	72	1	26	1	0
12/1/26	,,	9·3	32	81	3·2	6,500	63	5	28	3	1
13/1/26	,,	9·3	35	89	3·2	7,700	76	3	21	0	0
14/1/26	,,	8·2	30	79	2·9	6,700	71	1	26	1	1
18/1/26	,,	7·6	30	79	3·3	7,600	unable to count			—	—
19/1/26	,,	9·2	37	84	3·3	7,300	63	1	31	4	1
20/11/26	,,	10·0	39	81	3·0	6,000	unable to count			—	—
21/11/26	,,	9·3	34	84	3·0	8,600	69	2	25	3	1
22/1/26	,,	9·2	32	81	3·0	6,400	77	3	20	0	0
25/1/26	,,	8·0	27	79	3·0	5,700	76	2	20	1	1
26/1/26	,,	8·4	30	77	2·9	5,000	71	3	23	2	1
27/1/26	,,	9·1	30	80	3·0	8,900	68	1	28	3	0
28/1/26	,,	10·0	32	84	3·0	6,800	67	5	24	4	0
29/1/26	,,	10·0	36	87	3·0	7,100	72	4	22	0	2
1/2/26	,,	9·6	34	86	2·5	5,300	67	2	27	3	1
2/2/26	,,	9·0	32	77	2·2	4,900	77	0	21	2	0
2/2/26	,,	10·0	37	93	2·5	6,200	74	3	21	1	1
4/2/26	,,	9·0	32	84	2·4	6,400	70	1	23	6	0
5/2/26	,,	8·5	33	78	2·3	8,500	74	1	22	3	0
8/2/26	,,	9·6	32	80	2·4	10,300	55	0	39	5	1
9/2/26	,,	9·0	32	80	2·1	10,600	63	0	30	7	0
10/2/26	,,	10·0	37	96	2·4	7,300	unable to count			—	—
12/2/26	,,	10·2	34	90	2·5	5,900	56	0	34	8	2
15/2/26	,,	10·2	34	87	2·9	5,700	72	4	16	5	3
16/2/26	,,	8·4	29	76	2·9	6,000	62	2	32	3	1
17/2/26	,,	9·4	36	81	2·9	7,300	55	2	39	4	1
18/2/26	,,	9·3	30	84	3·0	8,700	78	0	14	5	3
19/2/26	,,	9·9	33	79	3·4	7,500	68	2	24	4	2
22/2/26	,,	10·7	35	95	3·1	7,000	68	2	25	2	3
23/2/26	,,	8·8	30	78	2·9	4,300	68	2	26	4	0
24/2/26	,,	9·5	32	84	3·0	5,100	75	4	18	1	2
25/2/26	,,	10·9	33	84	3·0	7,100	77	0	21	1	1
26/2/26	,,	9·1	30	75	2·8	6,600	83	0	16	0	1
1/3/26	,,	10·9	36	91	3·0	8,300	69	1	28	1	1
2/3/26	,,	9·3	30	80	2·9	5,500	69	5	24	2	0
3/3/26	,,	11·2	33	89	3·1	6,100	70	2	22	5	1
4/3/26	,,	11·0	35	90	3·1	8,500	66	3	26	3	2
8/3/26	,,	9·4	33	81	2·8	4,000	71	0	25	3	1
9/3/26	,,	11·7	34	83	2·8	6,900	68	1	23	4	4
10/3/26	,,	9·4	30	78	3·0	6,000	62	2	34	1	1

For abbreviations, see Table 1.
For method of calculating colour indices, see Table 4.

GRAPH 10.

SHEEP No. 13601.

Control Adult Sheep.

Normal Sheep.

Table 11.

SHEEP No. 11814.

Recovery Case.

Date.	Source.	R.C.	R.P.	Hglb. %	Viscosity.	W.C.	L.	M.	N.	E.	B.
3/11/25	Jugular	13·8	39	96	—	13,300	58	2	35	4	1
5/11/25	,,	14·5	39	99	—	10,600	55	3	41	1	0
9/11/25	,,	12·8	35	91	—	6,700	70	3	27	0	0
11/11/25	,,	14·4	40	95	—	7,600	53	2	44	1	0
17/11/25	,,	14·4	41	100	—	8,000	58	6	35	1	0
23/11/25	,,	13·9	37	96	—	10,100	63	5	31	1	0
30/11/25	,,	14·2	40	100	—	10,700	54	3	38	3	2
14/12/25	,,	13·4	37	95	—	10,300	52	1	38	9	0
28/12/25	,,	13·8	—	96	—	12,200	46	3	49	2	0
5/1/26	,,	10·8	32	77	3·2	17,700	47	1	40	12	0
11/1/26	,,	9·2	27	69	3·0	9,700	48	2	37	8	5
18/1/26	,,	9·7	25	65	3·0	8,100	—	—	—	—	—
1/2/26	,,	8·1	25	57	2·3	12,000	52	4	37	6	1
5/2/26	,,	7·1	21	54	2·2	9,400	49	3	42	2	4
8/2/26	,,	7·4	24	57	2·2	11,600	60	2	28	6	4
15/2/26	,,	7·6	—	58	2·8	11,800	58	3	29	9	1
22/2/26	,,	6·6	17	49	2·6	8,900	52	2	37	5	4
1/3/26	,,	7·6	24	57	2·6	11,200	44	2	45	6	3
8/3/26	,,	6·4	22	51	2·5	10,400	41	2	44	9	4
16/3/26	,,	—	23	52	2·7	11,800	43	6	43	6	0
22/3/26	,,	7·3	23	52	2·1	7,100	36	5	45	10	4
29/3/26	,,	7·6	23	55	2·6	12,900	37	3	54	4	2
8/4/26	,,	7·3	22	53	2·6	9,900	51	5	37	4	3
12/4/26	,,	7·6	23	50	2·7	10,700	46	5	38	11	0
26/4/26	,,	6·5	20	53	3·1	12,000	47	3	42	7	1
3/5/26	,,	—	25	58	3·1	12,900	45	0	40	10	5
10/5/26	,,	8·8	28	63	3·4	11,700	49	1	42	7	1
17/5/26	,,	9·9	32	75	3·6	15,200	35	2	60	3	0
26/5/26	,,	6·9	23	66	3·6	9,700	46	2	35	14	3
9/6/26	,,	8·6	26	65	3·4	12,800	47	4	31	16	2
23/6/26	,,	8·7	25	63	—	11,600	50	1	33	11	5
29/6/26	,,	8·5	27	60	3·5	11,000	54	3	34	7	2
26/7/26	,,	10·6	33	74	3·9	10,800	47	4	33	14	2
27/11/26	,,	12·2	37	96	—	13,700	47	5	39	9	0

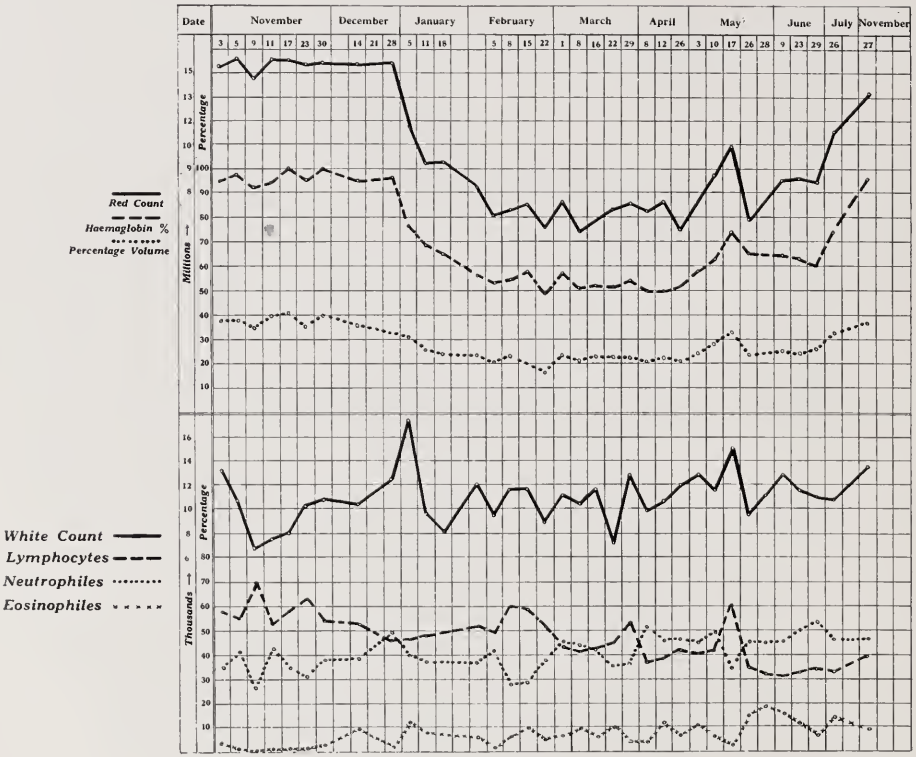
For abbreviations, see Table 1.
For method of calculating colour indices, see Table 4.

Table 10 and Graph 10 refer to Sheep No. 13601. This is an adult sheep also free from worms. The red count, haemoglobin and precipitate curves also show a normal variation, but the fluctuations are not so pronounced as in the case of the younger Sheep No. 11929, and within the limits of normal variation the curves are practically straight.

PATHOGENESIS OF THE ANAEMIA IN HAEMONCHOSIS OF SHEEP.

It is obvious that the outstanding pathological effect of wireworms on sheep is the very severe and often fatal anaemia that is produced, and it is of fundamental importance to establish definitely the manner in which this is brought about. It is remarkable that

GRAPH 11.
SHEEP No. 11814.
Recovery Case.



these parasites, spending as they do their entire parasitic life in the abomasum of sheep, can produce such profound changes in the blood of their hosts.

Theoretically, anaemia can be due to: (1) The destruction and disintegration of the red cells by (*a*) parasites such as occur in the piroplasmoses, or (*b*) toxic or other principles which can conveniently be classified as haemolytic substances; (2) A disturbance or disease of the blood-forming organs as a result of which the full ontogenetic development of the red cells is interfered with and the normal replacement of red cells cannot keep pace with those which are being daily eliminated in the process of normal wear and tear; (3) The removal of the red cells due to phagocytic activity of certain cells in the body. The anaemia is then due to excessive erythrophagocytosis. Since the reticulo-endothelial system plays an active part in the disintegration of red cells, e.g. in anaplasmosis in splenectomised sheep, according to de Kock (1923), erythrophagocytosis could perhaps more conveniently have been grouped and discussed under (1) above; (4) The removal of red cells as occurs in haemorrhage.

Before considering in detail into which of these groups the anaemia of haemonchosis can be placed from the known facts of the condition, and from the new evidence that is presented in this work, an attempt will be made to review very briefly, and without pretending to quote exhaustively the tremendous literature that has grown around this subject, the views generally held as to the manner in which parasites produce their effects on the hosts. In general the injurious effects of parasites on their hosts can be grouped into one or other of the following:—

(*A*) *Nutritive Disturbances.*—Either by depriving the host of food, which the gastro-intestinal parasites may assimilate, before this can become absorbed by the host, or by producing lesions in the alimentary canal, as a result of which large portions of the digestive tract are rendered valueless for digestion and absorption.

(*B*) *Mechanical injury inflicted during the migration of the parasites and bacterial infection through wounds and injuries.*—Such infection may have remote effects on various organs and tissues of the body.

(*C*) *Toxic Substances.*—There is the possibility that toxic substances, which are absorbed, may have a general or specific effect on the body as a whole, or on particular systems, such as the circulation, respiration, nervous system, endocrine organs, etc.

(*D*) *Anaemia.*—This may be due to (1) mechanical injury inflicted on the mucous membrane and underlying tissues by numerous parasites, particularly those equipped with mouth parts such as lancets designed for cutting, etc., and which secrete substances which prevent the coagulation of the blood; (2) the action of toxic principles, (*a*) haemolytic substances secreted by the parasites, which when absorbed will produce haemolysis, possibly also the action of haemolytic organisms which can gain entrance through mechanical injuries and wounds; (*b*) toxic substances which may cause injury to the blood-forming organs.

In considering to what extent any of these conditions are involved in haemonchosis of sheep and in some other parasitic diseases, as reported in the literature, they can be dealt with individually.

(A) No attempt was made to determine to what extent, if any, nutritive disturbances are associated with the injurious effects of wireworms. It is not likely that this can be an important factor in haemonchosis, as very often sheep which are heavily infested with wireworms are in fat condition, and it is doubtful if this deposition of fat can be entirely ascribed as secondary to the anaemia.

(B) The conditions enumerated under this heading may be involved on rare occasions in so far as haemonchosis is concerned, and will be referred to when some unusual lesions associated with wireworm infection are described subsequently.

(C) No such effects were observed or have been reported in the literature that was studied.

(D) Anaemia in Haemonchosis.

As previously stated, a systematic haematological study of the anaemia due to wireworm infestation in sheep is not recorded in the literature. On the question of toxic substances secreted by parasites, a vast literature has, however, accumulated.

Schwartz (1921) very fully discusses what he calls hematoxins from parasitic worms. He gives a comprehensive review of the literature, and mentions 75 papers. The period covered by this review is from 1865-1921. No attempt will be made to cover the same ground in the literature, but some of the conclusions in the author's summary of the recorded investigations on hemotoxins from parasitic worms, will be referred to. He states that: (1) certain parasitic worms secrete harmful toxic substances, which are named hemotoxins; these hemotoxins are in general of a non-specific nature and may be active on the blood of animals other than their normal hosts; (2) of the cestodes, *Diphyllobothrium latum*, definitely contains haemolytic substances; (3) *Schistostoma japonicum* and worms belonging to the genus *Ascaris* contain haemolysins; (4) hookworms, worms of the genus *Strongylus*, and *Gastrophilus* larvae secrete haemolysins and anticoagulins. *Haemonchus contortus* is specifically mentioned as apparently secreting a weak haemolysin and, further, whipworms as apparently secreting a haemolysin.

In concluding this summary, the author makes this statement: "Owing to the fact that the direct abstraction of blood by parasites appears to be inadequate as an explanation of the causes of anaemia in parasitic diseases, and in view of the fact that in tapeworm infections, which are accompanied by anaemia due entirely to the presence of the parasites, the direct abstraction theory is inapplicable, the view that haemolysins from parasites are of aetiological significance in parasitic diseases appeared to be entirely justified."

In his own work on salt solution extracts of *Haemonchus contortus* on sheep and cattle erythrocytes, Schwartz (1921) concludes that the weakly positive haemolytic effects obtained do not favour very strongly the view which has been commonly accepted as regards the secretion of a haemolysin by this parasite.

Hookworm disease in man and some of the domesticated animals has been extensively studied and, although Schwartz, just previously quoted, concludes from his study of the literature that haemolysins and anticoagulins are present in hookworms, Flue (1922) submits evidence, in connection with hookworm disease of dogs, from which he deduces: (1) the presence of anticoagulins in the cranial portions of these hookworms; (2) the absence of haemolytic substances; (3) that the worms are able to produce not only chronic, but also profuse haemorrhage, which is sometimes fatal. He further concludes that these facts favour the theory of chronic haemorrhage as the cause of anaemia in hookworm disease of dogs.

Recently Chandler (1929) states that it is generally agreed that hookworms do not cause anaemia by the secretion of a haemolysin. Hookworms suck blood, and as a result of the action of anticoagulins, the wounds keep bleeding for some time after the worms have abandoned them; but the amount of blood in the faeces, even in severe hookworm infestation, is usually very small. He concludes: "It seems fair to assume, therefore, that chronic loss of blood from the intestine is at most a minor factor in hookworm disease, and certainly cannot account for the severe anaemias so frequently seen in heavy infestations." He states further that "there seems to be little ground for doubting that the anaemia is due primarily to a toxic effect on the blood-forming organs."

Chandler quotes de Lange as stating that hookworm anaemia is primarily an aplastic one, caused by loss of normal regenerative power on the part of the blood-forming organs, due to toxins derived directly or indirectly from the worms in the intestine.

Nicoll (1914) maintains that hookworm disease in dogs "is generally accompanied by distinct though not profuse haemorrhage, which is most marked in the early stages, but tends to disappear. Evidence of blood regeneration was furnished by the appearance of large numbers of erythroblasts (normoblasts), which increased with the progress of the disease."

Mhaskar (1924) discusses the hookworm problem very fully, with the exception of the pathology and haematology of the condition. He uses the Duncan Whytes Phenolphthalein test to determine the presence of occult blood, and finds that: (1) occult blood is absent in cases free from hookworm infection; (2) it is present in 39 per cent. of infected cases; (3) it is present in 17.8 per cent. of cases for periods varying from 12 days to 4 months after the complete expulsion of hookworms; (4) the intensity of the reaction has no correlation to the number of hookworms harboured in the intestine.

All the cases examined were carefully selected and were free from all causes known to lead to evacuation of blood with faeces. It was supposed that the occult blood detected was due to haemorrhage consequent upon ulceration of the intestinal wall, brought about by hookworms, but the author states that he had no direct proof of this.

Scott (1930) believes that some of the acute anaemias in hookworm disease may be due to haemorrhage, but that the chronic anaemia does not seem to be fully explained on the basis of blood

loss. The remaining explanation, then, is a toxic depressing action on the bonemarrow and other blood-forming organs, but he points out that the pathology of these organs in chronic cases has not been adequately studied.

Referring now to the available literature in connection with the anaemia of haemonchosis in sheep, Hall (1920) mentions that the stomach worms of sheep have the habit of attaching at one point for a time and then moving away and attaching at another point, leaving the old point of attachment bleeding for some time. This causes anaemia, dry wool, oedematous swellings, and general unthriftiness. The anaemia produced by stomach worms resembles that caused by hookworms in man, dog, and sheep. The only way to differentiate between the anaemia of hookworm and wireworm infections in sheep is by post-mortem examination.

Donatien and Lestoquard state that anaemia of sheep and goats may to-day be attributed to (1) worms, (2) blood parasites, (3) bacteria, (4) pernicious anaemia. Group (1) is discussed as parasitic anaemia which can be caused by (*a*) distomiasis, (*b*) gastro-intestinal strongylosis which is a progressive anaemia, with loss of appetite and diarrhoea. The manner in which this anaemia develops is not discussed, neither is any evidence submitted in regard to changes produced in the blood by uncomplicated infection with the individual strongyles; (*c*) trichocephalosis, and (*d*) ovine bunostomiasis.

Hutyra and Marek (1926) state that the *strongylidae* bore into the gastric mucosa and suck blood from it. In this way they disturb the nutrition of the host to a degree proportionate to their number. More detrimental probably than the loss of blood is, however, the absorption of toxic metabolic products of the parasites.

The conclusion that one comes to in studying the literature on hookworm disease is that in spite of the great deal of work that has been done, it is not definitely known what the pathogenesis of the anaemia is. It seems to be generally agreed that direct haemolysis does not occur. There are quite a number of workers who believe that the anaemia is due to haemorrhage from the intestinal tract, but others maintain that, although this may be a somewhat insignificant contributory factor, the anaemia is mainly caused by the action of toxic products from the parasites themselves, or toxic bacterial products, or the combined action of these toxins on the blood-forming organs, although it is difficult to reconcile a depressing effect caused by toxins on the blood-forming organs with the leucocytosis, which is described by Sarless (1929) in some dogs experimentally infected *per os* with *Ankylostoma caninum*. Wintrobe (1931) suggests a classification of the anaemias on the basis of differences in the size and haemoglobin content of the red corpuscles. The anaemias in his Class 4 largely comprise those resulting from chronic blood loss. The characteristic feature of these anaemias, according to this author, is the decrease in the size of the red cells, accompanied by a relatively greater decrease in the haemoglobin content. On this account he suggests the term hypochromic anaemia. He found that the anaemia in hookworm disease is of this type. With such an anaemia one would expect to find a decrease in the colour, volume, and volume colour indices.

In the case of haemonchosis in sheep, the anaemia-due-to-haemorrhage theory has its adherents. Other workers state that haemorrhage, as well as toxins, causes the anaemia, but no evidence is adduced as to how exactly the toxins are supposed to produce this effect.

The four main causes of anaemia previously referred to, will now be individually examined, with the object of determining, from the available haematological and pathological facts that are already known or have been established in this investigation, into which of these groups the anaemia of haemonchosis can be classified.

(1) *The Destruction and Disintegration of the Red Cells.*

If haemolytic toxins from wireworms or bacterial products be absorbed, the degree of haemolysis that is produced will depend on the haemolytic potency of the toxin and on the amount of toxin absorbed. When haemolysis occurs the haemoglobin will be dissolved in the plasma and produce haemoglobinaemia and, depending on various factors, this may lead to haemoglobinuria and possibly to haemolytic icterus. Irrespective of whether the haemolysis is of such a degree as to produce haemoglobinuria or icterus, derivatives of haemoglobin such as occur in haemosiderosis, must be found in the tissues, especially in those cases where the haematological examination reveals a marked decrease over a short period of time in the number of the red cells and in the absolute haemoglobin content. There is no evidence of an erythrorhexis or an erythrolysis such as is described by de Kock (1923) for enzootic icterus and anaplasmosis in splenectomized sheep respectively.

How do the results obtained accord with these postulates? Clinical icterus or haemoglobinuria never occurs in haemonchosis. Haemoglobinaemia has never been observed. If haemolysis does occur, one would expect, especially in those cases where the red cells decrease to the extent of over 5 millions per c.mm. of blood within a week, that haemoglobinaemia could be diagnosed by examining the plasma after centrifuging the blood. This was always very carefully done, and on no occasion was evidence of haemoglobinaemia observed.

In all the fatal experimental cases of haemonchosis, the liver, lung, spleen, and kidney were specifically stained for iron but none could be demonstrated in these organs, with the exception of the spleen. A good deal of iron-containing pigment is normally present in the spleen, but in this organ, which in quite a number of cases was very much atrophied, it was even difficult to demonstrate the presence of any iron-containing pigment at all. It can, therefore, be definitely stated that no haemolysis occurs which can explain the anaemia in haemonchosis in sheep. It is generally agreed that this holds for hookworm disease also.

(2) *A Disturbance or Disease of the Blood-forming Organs.*

If this occurs, one must assume that the blood-forming organs will reveal some morphological evidence of such disturbance and sometimes, at any rate, a compensatory myeloid metaplasia in organs like the spleen and the liver, as occurs in lymphomatosis, leucaemia,

and in carcinoma of the bonemarrow, according to Naegeli (1923), and as occurs in lymphatic aleucaemia in the dog, described by Fourie and Ziehn (1930). It may, however, reasonably be argued that the toxins are carried in the bloodstream and their effect on the spleen and the liver would also be such as to prevent the development of any myeloid metaplasia.

In all the fatal cases of haemonchosis, the bonemarrow, including the fat bonemarrow, was carefully examined histologically and the only changes that were observed were those not of decreased activity but rather those suggestive of increased activity. Numerous neutrophile and eosinophile myelocytes, as well as immature forms of erythrocytes, were easily recognized in the sections. In the fat bonemarrow the fat spaces were extensively replaced by myeloid tissue, in which young forms of erythrocytes were frequent and in which the other usual marrow cells, such as megakaryocytes, neutrophile and eosinophile myelocytes, etc., could be identified. The neutrophilia, which occurs in nearly all cases towards the end of the disease, is evidence of an actively functioning myeloid tissue. Should the animal live long enough, with such a severe anaemia in chronic cases one would expect to find eventually exhaustion of the blood-forming tissues, and the severe poikilocytosis and the "pessarformen" that occur in some cases may be evidence that such exhaustion is taking place, although Naegeli (1923) points out that poikilocytosis is of small diagnostic significance and is probably dependent on changes in isotonicity of the blood. In the cases studied, there was a fatal termination before well defined exhaustion occurred.

That compensation was taking place is proved by the myeloid hyperplasia of the fat bonemarrow, contrary to what one would expect where such a circulating toxin causing disturbances in the blood-forming organs is postulated. Such toxin should also tend to prevent the formation of any compensatory myeloid hyperplasia in the fat bonemarrow.

There is further definite evidence that, in a number of these fatal cases of haemonchosis, an increase in the size of the cells occurs and, according to Naegeli (1923), the presence of small cells (microcytes) is the most significant and distinctive feature of a bonemarrow insufficiency (Knochenmarksinsuffizienz). Schilling (1922) describes microcytes as degenerative forms of erythrocytes which indicate degenerative changes in the bonemarrow.

It can, therefore, be concluded that, in the fatal cases of haemonchosis studied, there is no evidence of a disturbance in the blood-forming organs interfering with the normal replacement of red cells, which are daily being eliminated in the process of normal wear and tear. There is certainly a disturbance in the blood-forming organs, but this is caused by and is secondary to the anaemia and not the primary cause of it.

The possibility that the lesions described in the liver and other organs can be caused by toxic substances, will be discussed under section (4) below.

(3) *Erythrophagocytosis.*

Only on very rare occasions were cells, in which erythrophagocytosis had occurred, encountered; this, as a cause of the anaemia, can therefore be discarded.

(4) *Removal of the Red Cells as occurs with Haemorrhage.*

The mouth parts and the buccal capsule of wireworms are not developed to the same extent as in hookworms, but nevertheless these parasites cut and lacerate the mucous membrane and suck blood. One hundred live wireworms were collected from a sheep that was destroyed, were carefully washed in saline, then transferred to and washed in distilled water, and finally transferred to a third receptacle, also containing distilled water. In this they were ground up and, after filtration, the extract was examined spectroscopically, when typical haemoglobin bands were identified. Hall, previously quoted, states that when the parasite detaches, as it has a habit of doing, it leaves the old point of attachment bleeding for some time, and when hundreds of worms are present in heavily infested individuals, it is not inconceivable that serious effects will be produced.

In typical and fatal cases of haemonchosis, the abomasal contents are of a brownish chocolate colour, and are strongly positive for haemoglobin, when tested with tincture of guaiaconic acid. Positive tests with this reagent may even be obtained with the intestinal contents. If one takes into consideration the large amount of food and water that normally pass through the abomasum a great deal of blood must continuously flow into the abomasum in order to produce this discolouration of the abomasal and even the intestinal contents.

In most cases the anaemia begins to develop from the third to the sixth week after artificial infection. If it is considered that it takes three weeks for the parasites to become adult and allowance is made for an initial stimulating effect resulting in an increase in the number of red cells, it seems doubtful that toxins could produce the anaemia in such a short period of time. Owing to the relatively few cases in which a progressive anaemia developed, no attempt was made to destroy the parasites in an animal in the later stages of the disease, in order systematically to trace the regenerative changes in the blood during recovery, but from practical experience it can be stated that infested animals nearly always recover and rapidly improve after appropriate medicinal treatment. It seems unlikely that a bone-marrow, which has been so severely damaged by toxins that it cannot maintain even two million cells per c.mm. of blood, should immediately the toxins are removed be capable of producing sufficient cells to meet the ordinary requirements of the body.

It is now necessary to consider whether or not (1) the morphological changes in the red cells, (2) the changes in the various indices recorded, and (3) the pathological lesions described support the contention, that the anaemia is due to haemorrhage along the alimentary canal.

(1) *Morphological Changes.*

These are, briefly, poikilocytosis, polychromasia, punctate basophilia, and "pessarformen."

Poikilocytosis.—It seems to be generally agreed that this condition is of no great diagnostic value, although Piney (1927) regards it as a more definitely abnormal mode of regeneration that is anisocytosis, as it never occurs in normal individuals.

Polychromasia.—Naegeli (1923), Schilling (1922), Krumbhaar (1928), and other authors recognize this as a sign of youth of the cell, and as such it must be associated with an actively functioning bonemarrow. Piney (1927) states that polychromasia is simply a condition of slight immaturity of the red corpuscles.

Punctate basophilia.—Naegeli and other authors maintain that cells so affected are products of embryological or pathological reactions on the part of the bonemarrow, and are clinical manifestations of pathological regenerative changes; but Krumbhaar states that " it is still doubtful if they are signs of youth or, as most authorities believe, of degeneration." The same author quotes Lehmann, who believes that basophile granules may be manifestations of either condition.

As punctate basophilia is absent from the blood in aplastic anaemia, Piney (1927) concludes that it is of regenerative significance, but the fact that the cells showing this do not occur in embryonic blood indicates that they are the products of abnormal regeneration.

" Pessarformen."—These are large pale cells, which according to Naegeli indicate degenerative forms or an insufficiency in the formation of red cells in the bonemarrow. Such cells occur in the so-called secondary anaemias, and in those stages of haemonchosis when the limits of compensation have been reached. The presence of such cells may possibly be explained by the haemoglobin deficiency developed on account of removal of blood along the alimentary canal.

(2) *Changes in the Volume Index, Colour Index, and Volume Colour Index.*

In a number of cases there is a definite increase in the volume index and in the colour index. The increase in these indices is due to an increase in the size of the red cells. Some of these large cells are fully haemoglobiniferous and can appropriately be classified as megalocytes. Many of the macrocytes show polychromatic staining and others contain punctate basophilic granules. These macrocytes must be regarded as young cells and are the output of an actively functioning bonemarrow, which is reaching the limits of compensation, and is no longer capable of turning out fully mature cells, with the result that young cells must be pushed into the circulation. That the blood-forming organs are showing signs of exhaustion in the later stages of the disease is confirmed by the decrease in the volume colour index, and by the presence of pale cells, both of which indicate that the cells are deficient in haemoglobin. These changes in the morphology of the red cells and in the various indices are exactly what one would expect when internal haemorrhage occurs. On referring to Table 12 below, it will be seen that almost identical changes occurred in the red cells of a sheep which had been bled from the jugular vein until a severe anaemia developed.

Table 12.

SHEEP No. 20715.

Bled from jugular vein until anaemia developed.

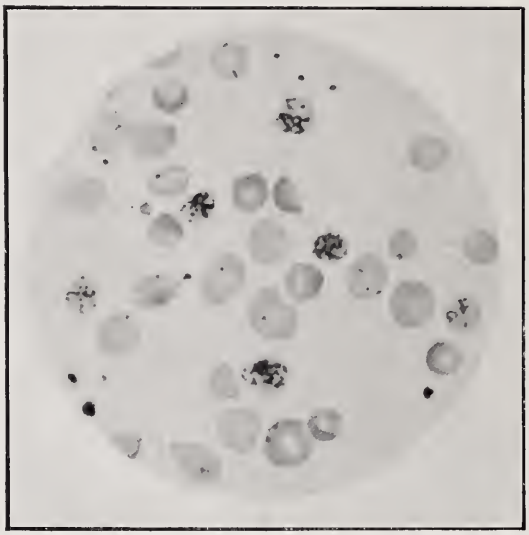
Date.	Source.	R.C.	R.P.	Hglb. %	W.C.	V.I.	C.I.	V.C.I.		Remarks.
4 11 40	Jugular	13·2	46	113	9,200	1 00	1·00	1·00	bled 600 c.cs.	—
5 11 30	,,	10·5	40	99	7,500	1·09	1·10	1·00	,, 600 ,,	—
6 11 30	,,	8·3	29	81	10,900	1·00	1·17	1·11	,, 500 ,,	—
7 11 30	,,	6·0	21	—	9,700	—	—	—	,, 400 ,,	—
8 11 30	,,	5·6	19	52	7,300	0·97	1·13	1·06	,, 400 ,,	—
10 11 30	,,	4·6	18	51*	4,700	1·12	1·35	1·15	,, 500 ,,	Some megalocytes present.
11 11 30	,,	4·0	15	41	8,000	1·04	1·20	1·11	,, 200 ,,	Cells are well preserved, megalocytes present.
12/11 30	,,	2·7	12	33	8,000	1·27	1·51	1·12	,, 200 ,,	No smears.
13 11 30	,,	3·3	15	41	8,200	1·30	1·56	1·11	not bled	Microcytes, macrocytes, polychromasia, and punctate basophilia.
14 11/30	,,	3·6	16	42	9,700	1·28	1·36	1·07	bled 400 c.cs.	Many large cells less marked, polychromasia and punctate basophilia.
15 11 30	,,	2·9	15	38	9,700	1·48	1·53	1·03	,, 200 ,,	No smears made.
17 11 30	,,	3·5	16	43	7,900	1·30	1·44	1·09	not bled	Reticulocytes with vital staining.
18/11 30	,,	3·7	17	45	4,100	1·32	1·42	1·08	killed	Punctate basophilia and polychromasia, reticulocytes numerous.

* Hemoglobin determination not reliable.
For abbreviations, see Table 1.
For method of calculating colour indices, see Table 4.

This sheep was bled almost every day, up to 600 c.cm. of blood being removed from the jugular vein at a time. Although large cells such as megalocytes were easily recognized microscopically previous to 13.11.29, macrocytes with polychromasia and punctate basophilia were for the first time numerous on this date, cells measuring up to 8.1μ being present. On 17.11.29, the blood was vitally stained and numerous reticulocytes were recognized. Just before the animal was destroyed on 18.11.29, the blood was again vitally stained, and the reticulocytes were exceedingly numerous on this date. The coverslips were removed from some of these vitally stained preparations, the blood was spread on the slide and after quick drying was stained in

Fig. 14.

SHEEP No. 20715.



×1350

Reticulocytes with vital staining.

the usual way with Giemsa. In these permanent preparations, a microphotograph of which is reproduced (Fig. 14), no polychromasia is present, but the reticulocytes are numerous. In the ordinary smears, stained by Giemsa, there are numerous cells showing polychromasia, and it would seem that the polychromatic cells in smears not vitally stained are the same as the reticulocytes in the vitally stained smears. This would tend to support those haematologists, such as Ferrata (1909), Schilling (1922), and others quoted by Krumbhaar (1928), who believe that the " vitally stained reticulum is an artificial clumping of the same substance that with Romanowsky methods stains diffusely as polychromatophilia."

The colour index and the volume index in this case of external bleeding is definitely increased. This is due, as in haemonchosis, to an increase in the size of the red cells. The volume colour index remains normal, whereas in haemonchosis it is decreased. If the animal could have been bled over a longer period of time, a deficiency in haemoglobin, with a decrease in the volume colour index would probably also have occurred.

It is noteworthy that the colour index, the volume index, and the volume colour index in some of these cases of haemonchosis are exactly like those of pernicious anaemia in the human subject. There is also a similarity in the morphological changes that occur in the red cells, e.g. anisocytosis, poikilocytosis, polychromasia and punctate basophilia, although undoubted megaloblasts, as such, were never identified in cases of haemonchosis. In pernicious anaemia there is a leucopenia and a relative lymphocytosis and Piney (1927) regards this as evidence of defective myeloid function. There is, on the contrary, a tendency towards a leucocytosis in haemonchosis, which would tend to support the view that in haemonchosis the bonemarrow is not defective.

(3) *Pathological Changes.*

Except for the myeloid hyperplasia in the fat bonemarrow and other evidence of active erythropoiesis, the lesions in pernicious anaemia of the human subject are entirely dissimilar to those of haemonchosis in sheep. The erythrophagocytosis, myeloid metaplasia, and haemosiderosis, which occur in pernicious anaemia, find no counterpart in the lesions of haemonchosis. From this it can be concluded that the injury to or the disturbance in the red cells must be different in the two conditions.

The lesions that are most consistently encountered in haemonchosis of sheep are (1) lesions resembling necrosis around the central veins of the liver, and (2) the presence in the cells around the central veins of substances which stain with Sudan III and with Nile Blue Sulphate, but which are soluble in alcohol; and further the presence in some of the cells, not only of the liver, but also of most of the organs of the body, of peculiar crystals, which resemble those which occur in fat necrosis. The presence of these substances, which are not true fats but probably related substances, may be associated with changes in fat metabolism in the liver and elsewhere. All these lesions are probably dependent on circulatory and nutritional disturbances, due to the anaemia. The peculiar distribution of the lesions just around the central veins of the liver suggest this. Owing to the anaemia, the total amount of oxygen is deficient. In the case of the liver, those parts first reached by the blood will readily absorb much of the already depleted available oxygen, and by the time the blood has flowed to the region of the central veins, sufficient oxygen is no longer available, and these peculiar lesions of characteristic distribution develop. MacCallum (1922) mentions a number of conditions of the liver, e.g., chronic passive congestion, chloroform poisoning, etc., where the lesions are confined to the cells around the central veins, and he makes the statement that " one cannot doubt the determining influence of the bloodstream in producing these

differences of distribution, although it is not always easy to understand it." This author believes that, in chronic venous congestion, the peculiar distribution of the lesions around the central veins can probably be explained on the basis of a circulatory disturbance, and that those cells which receive the blood last are poorly nourished and do not receive the proper supply of oxygen. It is nevertheless of importance to consider whether such lesions can be produced by toxic substances circulating in the bloodstream. In this connection one would expect that those portions of the liver, namely at the periphery of the lobules, which are first reached by the blood, should show, in a greater measure, the effects of the injury; but Opie, quoted by

SHEEP No. 15786: SPEC. No. 6833.
FIG. 15.

×450.

Crystals in Cells of Liver.

MacCallum (1922), maintains that in intense infections, and especially where a toxic injury is combined with bacterial infection, necrosis may occur in a part of the lobule midway between its periphery and the central vein. It seems, therefore, that on the basis of the distribution of the lesions alone, the possibility of toxic action cannot be absolutely excluded.

The crystals described are not specific for wireworm infestation. They are present in the cells of the livers from sheep which had been splenectomized by De Kock and Quilan (1926), and which subsequently developed anaemia due to anaplasmosis. They are also present in the liver cells of sheep that were bled until a severe degree of anaemia developed. The section shown in Fig. 15 is from such a

case. Their presence seems to be consistently associated with anaemia of the sheep, although they were also recognized in the liver of some cases of heartwater, but were absent in other cases of this disease. It is, however, not by any means certain that all the sheep which died from heartwater were entirely free from parasitic infestation. The crystals were demonstrable in organs which were cut and examined within a few days after formalin fixation and also when these organs, after preliminary fixation in formalin, had been kept for months in Kaiserling. That they are not artefacts produced in the Kaiserling is further proved by their absence from the cells of many other organs that were so preserved.

It can, therefore, be concluded that: (1) the wireworms ingest blood and pierce and cut the mucous membrane and underlying tissues of the abomasum, as a result of which there is constant haemorrhage into the abomasum. This causes discolouration of the abomasal and intestinal contents.

(2) There is a rapid production of the anaemia in severe cases of wireworm infestation, with prompt and complete recovery when the parasites have been destroyed.

(3) The anisocytosis, the polychromasia, the Jolly bodies and probably also the punctate basophilia, the active erythropoiesis with myeloid hyperplasia, the plus colour index, and the plus volume index indicate active regenerative changes and must be associated with an actively functioning bonemarrow.

(4) The poikilocytosis, the pale cells (pessarformen), and the decrease in the volume colour index indicate commencing exhaustion of the blood-forming tissues.

(5) The lesions in the liver and elsewhere are probably due to circulatory and nutritional disturbances and changes in fat metabolism, dependent on the anaemia, but the possibility that toxins may produce such lesions cannot be entirely excluded.

(6) With the exception of the lesions, like those of necrosis, occurring around the central veins and the decrease in the volume colour index, identical haematological and pathological changes are present in acute cases of *Haemonchus contortus* infestation in sheep, and in sheep bled daily from the jugular vein until a severe degree of anaemia has been produced.

In view, therefore, of the facts that: (*a*) there is no haemolysis, (*b*) there is no evidence of a primary disturbance in the blood-forming organs, which interferes with the normal replacement of red cells daily eliminated in the process of normal wear and tear, (*c*) there is very little, if any, erythrophagocytosis, and (*d*) there is positive evidence that the worms ingest blood and undoubtedly cause considerable haemorrhage into the abomasum, it is justifiable to conclude that the anaemia is due (1) to the haemorrhages which occur at the points where the worms attach and detach, and (2) to probably a much smaller extent to the ingestion of blood by the parasites.

When numerous parasites are present, a marked anaemia can be produced in a relatively short period of time. The initial effect of this removal of blood is to stimulate the blood-forming organs to

increased activity, resulting in a temporary polycythaemia or erythrocytosis. Very soon, however, a progressive decrease in the number of red cells takes place and when the bonemarrow can no longer meet these enormous demands for red cells, a compensatory myeloid hyperplasia, which also involves the fatty bonemarrow, takes place. Eventually a state of affairs is reached when the blood-forming organs cannot supply sufficient adult cells for the circulation, and young immature forms are released into the circulation. This explains the anisocytosis, the polychromasia, the Jolly bodies, the plus volume index, the plus colour index, and probably also the punctate basophilia. During the further course of the disease, however, the bonemarrow gradually becomes exhausted, degenerative forms, such as poikilocytes and pale cells, make their appearance, and with the enormous elimination of haemoglobin along the alimentary canal, there is eventually also a haemoglobin deficiency in the cells, as is indicated by the decrease in the volume colour index. As a result of the anaemia there is probably a disturbance in the fat metabolism of the body. This leads to the appearance of related fatty substances and crystals in the cells of various organs, but particularly of the liver. Further, lesions like those of necrosis, dependent probably on circulatory and nutritional disturbances due to the anaemia, develop around the central veins of the liver.

That somewhat similar lesions and practically identical haematological changes occur when sheep are bled from the jugular vein until a severe degree of anaemia develops is very strong confirmatory evidence that the changes in the blood picture of haemonchosis in sheep are not due to a primary disturbance in the bonemarrow, but are the result of, and secondary to, the anaemia.

SOME UNUSUAL LESIONS OF HAEMONCHOSIS.

On three occasions infarcts were found to be present in the hearts of sheep that had a severe wireworm infestation. In the one case an embolus or thrombus was located in the left coronary artery, in which it formed a nodule that could easily be felt with the finger. On the vessel being opened and embolus or thrombus removed, two smaller branches of the left coronary artery were found to be completely occluded at this point, and that portion of the myocard supplied by these two branches was of a dark red colour. The area involved in the infraction measured 5 by 2 cm. (Fig. 16). No emboli or thrombi could be detected in the lungs. The valves and endocardium of both the left and right portions of the heart were in no way diseased. In this case the origin of the emboli could therefore not be traced. Even theoretically it is difficult to conceive in what way the wireworm infestation, or even the anaemia produced by it, could cause the formation of emboli or thrombi in the coronary arteries.

In this same animal there were numerous ulcers in the abomasum. In many cases, as will be seen from the accompanying photograph (Fig. 17), numerous wireworms are attached to these ulcers. In some cases the ulcers are elliptical and measure as much as 3 by 2 cm., in other cases they are very irregular in outline. The depth to which the ulcers extend into the wall of the stomach varies very much:

FIG. 16.—SPECIMEN No. 9461.

1. Left coronary artery.
2. Branches of left coronary artery completely occluded.
3. Infarction resulting from occlusion of vessels. (See 2.)

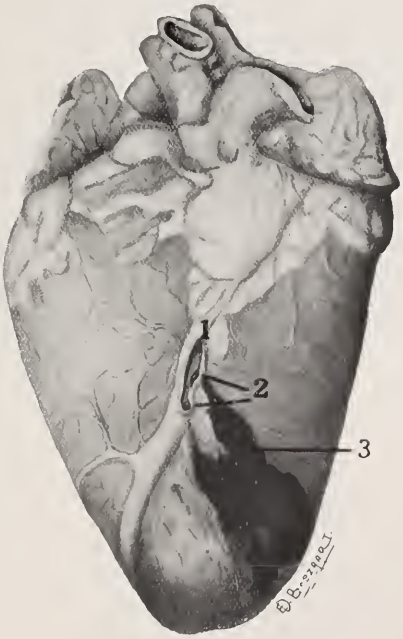


FIG. 17.—SPECIMEN No. 9461.

1. Wireworms attached to ulcers.
2. Ulcers.

some are superficial while others penetrate deeper into the submucosa, and in the more severe cases they may even extend through the muscular layers and are then apparently only limited by the serosa.

I am not aware that the view has ever been advanced that wireworms can be responsible for ulcers in the abomasum. It seems probable that the injury inflicted on the mucous membrane by these parasites may, in some cases, upset its normal protective mechanism, and there may then develop peptic digestion of the stomach wall; or certain bacteria may be responsible for the destruction of the tissues in a secondary manner. That the worms themselves destroy the tissues and cause the ulcers is most unlikely. It is more difficult to understand the pathogenesis of the infarction of the heart. No thrombi could be detected in the lungs, nor were the valves and endocardium in any way diseased. It is possible that bacteria gained entrance to the circulation through the ulcers in the abomasum, and were able to produce injury to the intima of the coronary artery, with thrombus formation in a heart whose resistance was decreased as a result of the anaemia. In anaemia the heart is undoubtedly called upon to do a great deal of extra work, as the smaller number of red cells must circulate so much more frequently in order to supply the necessary amount of oxygen. This would occur in spite of the presence of certain compensatory factors. It is, for example, well known that sheep with severe anaemia from wireworm infestation are easily fatigued; and the probalitiy is that these animals are less active than normal, and consequently do not require the same amount of oxygen as do normal animals.

SUMMARY.

(1) Sheep blood takes longer to precipitate than horse blood When centrifuging normal citrated sheep blood in an electric centrifuge running at 1,500 revolutions per minute, one obtains constant readings in 60-70 minutes time. Under similar conditions, the blood of sheep with severe anaemia will give constant readings in 20-30 minutes.

(2) Uniform relative results are obtained when haemoglobin determinations on sheep blood are made in a Dubosq colorimeter, using a Newcomer disc as standard.

(3) In order that reliable colour, volume, and volume colour indices may be calculated, it is advocated that normality for each individual should first of all be established during the pre-infection period.

(4) Of 38 worm-free sheep that were artificially infected with pure faeces cultures of *Haemonchus contortus* larvae, 9 developed a fatal progressive anaemia, and 6 developed anaemia from which they recovered without any medicinal treatment (so-called "recovery cases"). One further recovery case resisted repeated attempts at infestation with large doses of wireworm larvae. The remaining 23 sheep either resisted infection or did not show any effects of infestation.

(5) In haemonchosis there is (*a*) no icterus, haemoglobinaemia or haemoglobinuria, because haemolysis does not occur; (*b*) no evidence of a primary disturbance in the blood-forming organs, interfering with the normal replacement of red cells which daily are being eliminated in the process of normal wear and tear. Any disturbance that is present in the bonemarrow is secondary to the anaemia; and (*c*) very little, if any, erythrophagocytosis.

(6) The wireworms ingest blood and cause haemorrhage into the abomasum, resulting in a chocolate discolouration of the abomasal contents. This haemorrhage and the ingestion of blood by the parasites is the cause of the anaemia, which shows the following characteristics: (*a*) there is probably an initial polycythaemia or more particularly an erythrocytosis due to the stimulating effect produced by the haemorrhage, etc. (*b*) Regenerative forms of erythrocytes are present, e.g., cells showing anisocytosis, polychromasia and Jolly bodies. Punctate basophilia is also present and can probably also be included with regenerative forms. When such regenerative forms and megalocytes predominate, there is an increase in the colour index and the volume index, due mainly to the increase in the size of the red cells. (*c*) Degenerative forms are present, e.g., pale cells (pessaformen), poikilocytes, and possibly punctate basophilic cells. These indicate commencing exhaustion of the blood-forming organs and associated with them is a decrease in the volume colour (saturation) index, pointing to a relative deficiency in haemoglobin.

(7) No lesions which can be regarded as specific for haemonchosis are present. A myeloid hyperplasia is present which also involves the fat bonemarrow, and is secondary to the anaemia. Lesions like those of necrosis immediately around the central veins and the presence of related fatty substances and crystals in the cells of the liver, as well as in cells of other organs, are probably due to circulatory and nutritional disturbances, and to disturbances in the fat metabolism of the body as a result of the anaemia.

(8) With the exception of the lesions, like those of necrosis, occurring around the central veins of the liver, and the decrease in the volume colour index, identical haematological and pathological changes are present in acute cases of *Haemonchus contortus* infestation in sheep and in sheep bled daily from the jugular vein, until a severe degree of anaemia has been produced.

(9) There is a neutrophile leucocytosis towards the end of the disease.

(10) The colour index, the volume index, and the volume colour index of haemonchosis are exactly like those of many cases of pernicious anaemia in the human subject. The morphological changes of the red cells are very similar to those of the latter disease except that, in the fatal cases of haemonchosis studied, no megaloblasts were identified. The lesions, however, are entirely dissimilar, and haemosiderosis, erythrophagocytosis, and the myeloid metaplasia of pernicious anaemia are not present in haemonchosis. From this it is evident that the nature of the injury in or the disturbance to the red cells must be different in the two conditions.

(11) Some unusual lesions, e.g., infarcts in the heart and ulceration of the abomasum, were on rare occasions found in fatal cases of haemonchosis.

ACKNOWLEDGEMENTS.

This work was first of all suggested to me by Sir Arnold Theiler, the late Director of Veterinary Education and Research, Onderstepoort, Union Department of Agriculture.

I wish to record my thanks to my colleagues the late Dr. C. P. Neser and Dr. G. de Kock for valuable advice, and to Mr. P. C. van Niekerk, Senior Lay Assistant, for assistance in the haematological work.

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Studies on the Blood of Mice.

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The writer was afforded an opportunity of studying the blood of mice in the Mousery of the Department of Experimental Surgery (Director, Dr. Alexis Carrel), Rockefeller Institute, New York. Different strains (or races) of mice, e.g. Rockefeller, Swiss, Lathrop, Little, etc., are utilised to study the influence of diet, habitat, etc., in respect of resistance to disease. It was thought that the study of the blood of mice in these carefully controlled groups may offer an explanation as to why such great variations, especially in respect of total leucocytic count, occur in the blood of mice.

In this paper the observations made and the data collected will be presented under the following:—

(1) Consideration of the technique utilised in this study.
(2) A consideration of the race, age, diet, habitat, etc., of the mice studied.
(3) Interpretation and discussion of the data given in the Appendices I and II.
(4) Conclusions.
(5) Appendices I and II.

Technique.

Particular attention may be drawn to the following:—

(*a*) Collection of the blood;
(*b*) erythrocytic count;
(*c*) leucocytic count;
(*d*) differential counts:—
  (i) in fixed preparations,
  (ii) by the supravital method;
(*e*) grams of haemoglobin per 100 c.c. of blood;
(*f*) cultures of the buffy coat of the blood.

(*a*) The technique was mainly based on the one now in general use at the Onderstepoort Laboratory (Neser, 1923), and the one followed by Sabin (1923) and her co-workers.

In view of the fact that some authors believe that there exists a daily rhythm of total leucocytic counts, mice were bled at different hours during the day, e.g. 9-10, 10-11, 11-12, and 2-3. In the collection of the blood either from the *tail* or the *aorta*, particular attention was paid to the following, in order to see whether any of these were concerned with the variations referred to—

(i) *influence of an anæsthetic;*

(ii) *position of the animal* when being bled, i.e. whether it was standing, lying on its back, or on its abdomen.

Mice were therefore bled either from the tail or aorta, or both, with and without an anaesthetic. In some instances determinations were made from the tail and aorta of the same mouse. In others several counts from the tail of the same mouse were made at varying intervals, from a few minutes to several days. It was, however, found that, if the interval between first and second bleeding exceeded some time then the wound on the tail prevented a sufficient quantity of blood being obtained for subsequent counts, in spite of removing another ½-1 inch of the tail. Apparently damage to the blood vessels of the tail caused by the first bleeding prevented a regular flow of blood. For the count from the tail, mice were placed in the standing position in a small wire gauze cage, which allowed of as little movement as possible. The tail was passed through a small opening and allowed to hang in a basin of warm water 40°-45° C. for several minutes. The mouse in the interim had completely settled down and had become completely reconciled to its surroundings. The tail was then carefully dried without undue rubbing or massaging. With a sharp scissors about ½-1 inch of the extremity of the tail was removed by a sharp cut. From the first drops of blood the sample for (1) the total leucocytic count was made because it showed the greatest variation. Then samples of blood were collected for (2) enumeration of red cells, (3) estimation of the amount of haemoglobin, (4) differential counts: (i) supravitally stained, and (ii) in fixed preparations.

The same procedure of collecting blood was, as far as possible, carried out when blood was taken from the aorta. The influence of a general anaesthetic (ether) was also tested out in mice bled from the aorta and the tail. In the above method from the tail, mice were bled in the standing position, whereas the blood from the aorta had to be taken from the mouse being on its back. In order to see whether the position of the mouse influenced the total leucocytic count, a number of mice were secured either on their abdomen or back, by means of plaster strips and then bled from the tail.

(*b*) and (*c*) The dilution of the blood for the white and red counts was effected by pipettes used by Sabin, and the counts were made in a modified Bürker.

(*d*) The *fixed preparations* for the differential counts were made according to a slight modification of the method described by Neser. Glass slides (X) about three inches long and one inch broad, are used to make the smears on, and squarely cut portions of similar glass slides (Y), slightly larger than one inch square, were used instead of cover slips for spreading the blood. The glass slides, etc., used should be absolutely free from grease and thoroughly cleaned. With

a platinum loop of suitable size, a small drop of blood is taken as it issues from the blood vessel. It is then rapidly transferred to the glass slide (X) which is held in a horizontal position between the thumb and forefinger of the left hand. The drop is placed on the main axis of the slide, towards the end held between the fingers. The cut portion of the glass slide (Y) held broadwise between the thumb and the forefinger of the right hand, is then very quickly lowered over this drop, which spreads uniformly between the two surfaces. The cut portion of the glass slide (Y) still held lightly between the thumb and forefinger of the right hand is now drawn over the glass slide (X) along its length at such a rate that the resulting film is so thin that it presents rainbow colours. This method of preparing smears is easy and the leucocytes are as a rule evenly distributed throughout the smears and gave more uniform results than smears prepared in any other way.

The supravital method of Sabin (1923) was also utilised for making differential counts to control the differential counts of the fixed preparation. It was found that better staining results were obtained in the case of the blood of mice when less of the saturated Janus Green solution was used for the supravital preparations.

(*e*) For the estimation of *Haemoglobin* the Klett colorimeter with artificial illumination was used.

(*f*) It was not possible to evolve a method by means of which constant uniform results could be expected with *cultures* of the buffy coat of the blood of mice taken from the aorta. As was to be expected, great difficulty was experienced in obtaining a sufficient quantity of blood from the aorta, on account of the minute calibre of the vessel, the small amount of blood available, and the rapid coagulation of the blood. The blood was collected in (7) various ways in order to check the formation of thrombocytes as a milky white film around the edge of the culture. Blood from the aorta was either collected through an extremely thin oiled glass tube and centrifuged for 2-5 minutes in a thoroughly cooled centrifuge. Blood was also taken in Heparin (1-1,000 to 1-10,000) and centrifuged for 2-5 minutes. Coagulation was extremely rapid in the mouse, and often several tubes of blood had to be made before one with a buffy coat was obtained. The plasma was pipetted off and the buffy coat covered with a drop of embryonic extract from the chicken or the mouse. In some instances the tube was again centrifuged for a few seconds at 3,000 revolutions, before it was placed into an incubator from ten minutes to one hour. The whole coagulum in the tube was then carefully loosened from the tube by means of a platinum needle, and dropped into a small glass dish containing tyrode. The buffy coat was then separated from the coagulum and freed from erythrocytes. It was then transferred to a clean hollow glass slide and cut into fine pieces, about $\frac{1}{2}$-1 mm. square. In view of the indifferent results obtained in these cultures, a large combination of media were tried out, e.g. chicken plasma, rat plasma, or mouse plasma, concentrated or diluted with tyrode, and to these latanie, chicken embryo extract or mouse embryo extract was added. Cultures in Ringer-Heparin solutions were also tried.

Cultures were made on mica or large cover slips and the medium was spread very thinly. A small number of fragments were also cultivated in Carrel flasks. For vital staining a 1 : 1,000 neutral red stock solution was diluted 1 : 19 with tyrode. To the culture on the glass slide grown over a round hollow slide a few drops of the neutral red solution is added and over it a specially prepared hollowed out thin glass slide is placed, sealed and examined in the hot box. These hollowed-out glass slides were prepared in the following way: A thin slide with a circular hole about 1 cm. in its centre, is fixed to a thin cover slip by means of a drop of paraffin and gently heated so that the paraffin spreads evenly between cover slip and glass slide.

Fairly interesting preparations were obtained by subsequently fixing and counterstaining with Giemsa some of the supravital preparations used for observations in the hot box. Some of the cultures were stained with May-Grunwald-Giemsa, shortly after they were made, and others at intervals up to 48 hours. In this way an opportunity was offered for studying the various leucocytes and the thrombocytes which had migrated from the explant.

A Consideration of the Race, Diet, Habitat, etc., of the Mice Utilised in These Studies.

The mice, about 14,000 in number, are fed once a day, in the afternoon. Mice from various diets were selected. The value of these diets will be fully considered by Carrel in a subsequent paper. It may be pointed out here that an attempt was made to select diets with fairly wide variations, e.g.

Sherman + fat.
,, + sugar.
,, + meat.
,, + meat and potatoes.
,, + arsenic.
,, + salts.

The mice in all diets, except standard, have water present at all times by means of a tube from a bottle inverted on the cover of the box. The bottles are sterilised two or three times a week and the mouse boxes (10 by 7 by 5 cm.) are changed once a week. Boxes and covers are steamed for 20 minutes, dried, and filled to a depth of about 1½ inches with sterilised shavings. A temperature of 70° F. is maintained by thermostat during cold weather. In summer time the rooms are kept somewhat cool by window pressure fans, rotating fans, etc, Eight females and one male are kept in one box. When pregnant the females are transferred two to a box. At the age of four weeks the young are weaned and collected into breeding boxes. To save time and space a male is added or left at weaning, although mice do not mature until about the sixth week.

A number of mice were allowed to run free on ground laid down in fairly large compartments in a green house which was also more or less maintained at the same temperature. These mice, therefore, had a great deal of exercise and counts were made from these to study the influence of exercise. In the majority of the experiments non-pregnant females were used.

Rockefeller and Swiss strains of mice were selected because they were represented in most of the diets studied. The Rockefeller is a large white mouse, whereas the Swiss is somewhat smaller. Both strains were fairly well standardised. It may be pointed out here that in both strains a certain number of lung neoplasms (a type of papilliform cystadenomata) and mammary gland carcinomata were identified. Apart from lung and mammary gland tumours, other types of neoplasms in these strains were comparatively rare. Only a few cases of sarcomata were recorded. Lymphoid hyperplasias, especially associated with the spleen, liver, thymus, and lymphatic glands, were not infrequent in these strains, yet there was no evidence of a lymphatic leucaemia. A localised bronchitis was also seen in a few cases and macroscopically it was possible to confuse these latter changes in the lung for multiple lung neoplasms referred to above.

A lobular broncho-pneumonia, especially affecting the cardiac lobe of the right lung was seen in a number of mice in the mousery. Besides the above pathological changes a few cases with the following changes were also identified: localised alopecia, eczema, sarcoptes, mummification of foetuses, taenia, cirrhosis of the liver, kidney cysts, etc. In view of these findings in the Rockefeller and Swiss mice, it was decided to autopsy the majority of mice after they had been utilised for these blood studies, especially those in which excessive total leucocytic counts were recorded. In none of the mice, however, could anything of the nature of a lymphatic leucaemia be identified. *Mice of different ages were selected.* The following ranges were adopted: —

(*a*) Two, three and four months.
(*b*) Five, six, seven and eight months.
(*c*) One year.
(*d*) Two years.

In some instances mice one month old belonging to the same litter were utilised. Furthermore, in each group an attempt was made to select mice more or less of the same constitution and size, although variation in this respect was very slight, the Swiss and Rockefeller mice were found to be on the whole fairly uniform in the different groups.

Interpretation of the Numerical Data and Discussion.

Full particulars of the results obtained in various studies are given in the appendices as previously mentioned, the following were particularly stressed and controlled: —

(i) Time of bleeding;
(ii) collection of the blood sample in such a manner to exclude as far as possible any mechanical interference;
(iii) mice free of disease, of the same race or strain, the same age, the same sex and kept under the same environment were, as far as it was possible, selected;
(iv) in the various groups mice more or less of the same constitution were utilised.

In the appendices it will be seen that the following data were collected in the studies on the blood of mice:—

1. *Total leucocytic count*:
   - (*a*) from the tail;
   - (*b*) from the aorta;
   - (*c*) at various times of the day;
   - (*d*) at various ages;
   - (*e*) in various diets;
   - (*f*) of two strains: Rockefeller and Swiss.
2. *Differential counts*:
3. *Erythrocytic counts*:
4. *Grams of haemoglobin per 100 c.c. of blood.*

From the data it will at once be realised that it was not possible to explain why such variations, especially in total leucocytic count, were obtained in mice of the same group, seeing that every care was taken to exclude the various exogenous influences that may have operated.

It was pointed out that mechanical influence in the collection of the blood sample from the tail was as far as possible eliminated. The first drop of blood was utilised for total leucocytic count. This drop was obtained without exerting any rubbing or massage. The cut was sharp and sudden and yet, in spite of these precautions, fluctuations occurred which could, on the other hand, not be attributed to environmental influences.

That the sample of blood taken from the tail as indicated above, more or less represented the distribution of the leucocytes or " level " of leucocytes in the tail vessel at the time, cannot be doubted, if the data in Table I are scrutinised.

TABLE I.

Swiss Mice, 2-3-4 *Months.*

No. of Mouse.	Time of Bleeding.	Grams of Haemoglobin per 100 c.c. Blood.	Red Blood Cell Count.	White Blood Cell Count.
62K.....	11·0	—	—	11,900
62K.....	11·04	—	—	11,500
65K.....	11·35	—	—	10,060
65K.....	11·38	—	—	10,300
68K.....	11·00	—	—	24,100
68K.....	11·03	—	—	21,900
64K.....	2·35	15·8	—	8,200
64K.....	2·39	15·2	—	7,070
66K.....	1·45	19·3	—	10,400
66K.....	1·48	19·0	—	8,000
77K.....	1·25	—	—	9,700
77K.....	1·28	—	—	6,500
81K.....	2·15	—	—	17,800
81K.....	2·20	—	—	16,800

TABLE I—(*continued*).

No. of Mouse.	Time of Bleeding.	Grams of Haemoglobin per 100 c.c. Blood.	Red Blood Cell Count.	White Blood Cell Count.
		Swiss Mice, 5-6-7 Months.		
177K	9·25	18·7	—	12,900
177K	9·28	18·8	—	12,400
178K	9·40	18·5	—	10,200
178K	9·43	18·0	—	7,700
207K	9·25	20·5	—	14,900
207K	9·28	19·3	—	11,200
208K	9·45	20·7	—	13,500
208K	9·48	20·3	—	11,600
61K	10·05	19·6	—	25,400
61K	10·07	17·8	—	24,900
209K	10·25	22·5	—	14,700
209K	10·28	21·1	—	12,900
210K	10·35	20·5	—	13,300
210K	10·38	17·9	—	14,100
63K	11·48	15·3	—	16,600
63K	11·45	17·3	—	19,800
83K	11·30	19·9	—	12,600
83K	11·33	19·9	—	9,500
174K	11·25	22·9	—	12,300
174K	11·25	22·8	—	11,000
180K	11·10	19·0	—	10,800
180K	11·13	18·5	—	7,800
181K	11·20	20·3	—	15,200
181K	11·23	19·9	—	13,600
182K	11·35	20·5	—	4,200
182K	11·38	20·0	—	5,500
204K	11·30	—	—	12,700
204K	11·33	—	—	11,500
213K	11·15	22·3	—	20,000
213K	11·18	22·3	—	20,200
214K	11·25	21·1	—	18,000
214K	11·28	21·3	—	15,800
111K	1·50	20·3	—	15,100
111K	1·53	17·9	—	13,000
88K	1·50	18·2	—	13,500
88K	1·53	17·8	—	11,600
110K	1·53	16·6	—	14,000
110K	1·56	16·5	—	12,200
172K	1·50	19·4	—	18,400
172K	1·53	21·1	—	20,200
183K	1·45	18·7	—	16,100
183K	1·48	19·1	—	14,800
211K	1·50	21·3	—	17,900
211K	1·53	20·9	—	14,300
79K	2·33	—	—	9,700
79K	2·37	—	—	6,200
86K	2·45	—	—	15,800
86K	2·48	—	—	12,900
53K	2·20	20·5	—	5,900
53K	2·23	20·0	—	7,800
153K	2·30	—	—	12,800
153K	2·33	—	—	17,000
184K	2·60	20·5	—	7,200
184K	2·03	20·9	—	6,600
191K	2·26	19·8	—	18,900
191K	2·23	19·8,	—	14,800
197K	2·50	—	—	18,000

TABLE I—(*continued*).

No. of Mouse.	Time of Bleeding.	Grams of Haemoglobin per 100 c.c. Blood.	Red Blood Cell Count.	White Blood Cell Count.
197K.....	2·53	—	—	13,900
205K.....	2·35	16·5	—	4,800
205K.....	2·38	16·2	—	3,800
206K.....	2·55	20·5	—	4,500
206K.....	2·58	19·1	—	3,300
212K.....	2·05	19·6	—	22,200
212K.....	2·08	19·9	—	15,200
		Swiss Mice, 1 Year.		
100K.....	9·48	17·5	—	5,300
100K.....	9·51	17·5	—	5,400
126K.....	9·30	—	9·90	10,500
126K.....	—	—	8·03	6,600
52K.....	11·05	—	9·92	10,500
52K.....	11·55	—	9·5	10,000
102K.....	11 16	—	—	11,100
102K.....	11·13	—	—	9,600
43K.....	11·15	20·7	10·4	13,900
43K.....	11·20	19·8	10·5	9,350
89K.....	11·15	16·5	—	10,900
89K.....	11·18	15·2	—	10,300
96K.....	11·30	—	—	22,200
96K.....	11·33	—	—	21,400
95K.....	11·20	—	—	20,400
95K.....	11·23	—	—	21,600
87K.....	1·35	—	—	12,700
87K.....	1·38	—	—	8,500
93K.....	1·45	20·5	—	23,100
93K.....	1·48	19·1	—	19,800
94K.....	1·58	18·0	—	10,500
94K.....	2·01	17·2	—	10,500
146K.....	2·15	—	—	4,300
146K.....	2·18	—	—	4,100
45K.....	2·30	20·5	—	13,350
45K.....	2·35	18·5	—	12,100
		Swiss Mice, 2 Years.		
51K.....	10·50	16·8	—	10,300
51K.....	10·55	16·0	—	9,900
108K.....	10·45	—	—	39,400
108K.....	10·48	—	—	33,800
101K.....	10·50	20·7	—	7,000
101K.....	10·53	19·4	—	6,200
46K.....	11·25	17·0	—	20,500
46K.....	11·30	20·7	—	18,700
103K.....	2·10	—	—	13,200
103K.....	2·13	—	—	12,600
47K.....	2·10	—	—	12,600
47K.....	2·15	—	—	11,500
104K.....	2·25	—	—	9,000
104K.....	2·28	—	—	9,100
109K.....	2·23	15·3	—	18,300
109K.....	2·26	13·7	—	14,100
50K.....	2·55	16·2	—	10,300
50K.....	2·58	15·4	—	8,650

In the majority of these cases samples, from the same mouse taken at an interval of a few minutes, showed only slight differences. It will be seen that these differences in each individual mouse are well within certain "levels." This becomes apparent when some of the extreme levels given in Table I are compared:—

No. of Mouse.	Total Leucocyte Count.	
	First Bleeding.	Second Bleeding after a couple of Minutes.
1	21,400	21,000
2	8,200	7,900
3	17,800	16,800
4	25,400	24,900
5	4,200	5,500
6	20,600	20,200
7	18,400	20,200
8	5,900	7,800
9	4,800	3,800
10	5,300	5,400
11	22,200	21,000
12	39,400	33,800

Furthermore, it is interesting to note that the second sample of blood in the large majority of cases gave a lower total leucocyte count than the first. It would, therefore, appear that in total leucocytic counts mechanical factors apparently did not play an important part and that these fluctuations in the white cell counts of mice are not due to exogenous influences.

In view of these findings, it was therefore considered advisable to express total leucocytic counts of the mouse rather in "levels" than in "averages." This method of procedure is illustrated in Table II.

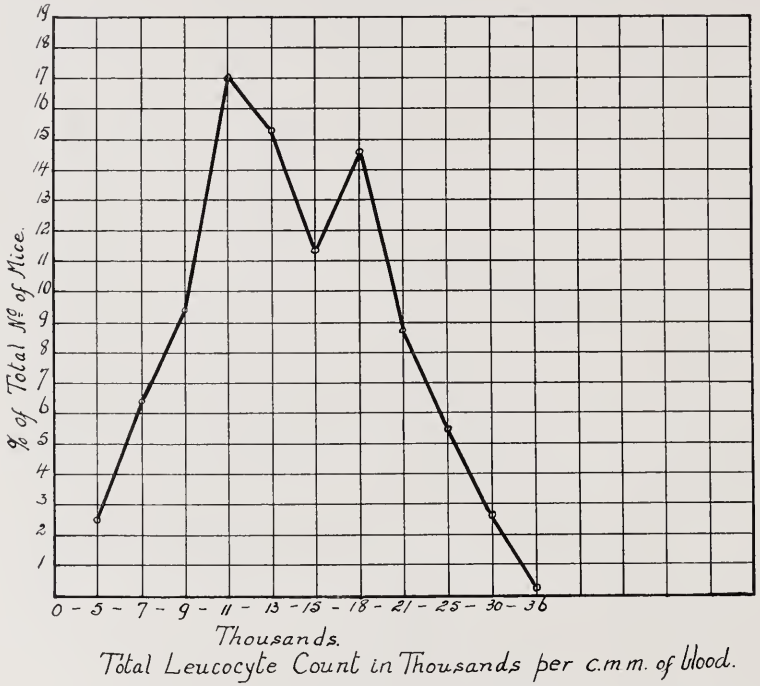
TABLE II.

No. of Leucocytes per cubic mm.	2–3–4 months.		5–6–7 months.		1 year.		2 years.		Total.		Total R+S.	%
	R.	S.	R.	S.	R.	S.	R.	S.	R.	S.		
–5000	—	—	—	6	—	2	—	—	—	8	8	2·6
5001–7000	—	3	2	8	2	3	—	2	4	16	20	6·5
7001–9000	1	5	3	8	7	4	—	1	11	18	29	9·5
9001–11000	2	7	6	13	7	12	—	6	15	38	53	17·0
11001–13000	1	4	9	21	4	5	—	3	14	33	47	15·3
13001–15000	1	5	8	24	7	7	—	3	16	39	55	11·4
15001–18000	3	6	7	19	6	4	—	—	16	29	45	14·6
18001–21000	—	3	1	14	—	2	—	4	1	23	24	7·8
21001–25000	—	3	2	6	—	6	—	—	2	15	17	5·5
25001–30000	—	3	—	2	—	2	—	1	—	8	8	2·6
30001–36000	—	1	—	—	—	—	—	—	—	1	1	·3

Abbreviations: R—Rockefeller mice.
S—Swiss mice.

In this table is indicated in the various columns the number of Rockefeller and Swiss mice of various ages, which gave total leucocytic counts according to the arbitrary levels suggested in column 1. If the percentages of the number of mice represented in these levels are compared, it will be seen that the total leucocytic count could be expressed in the form of a curve with the highest percentage between 11,000-18,000 leucocytes per c.mm.

CHART I.



From the data in the appendices, it will be seen that the total leucocyte count from the tail actually varied from about 4,000-39,000. A large number of mice which gave these extremes were afterwards carefully autopsied to ascertain whether mice with high level counts were not associated with a leucaemia or some other pathological alteration. It was, however, not possible to detect any specific pathological change in the autopsies conducted to explain these extreme counts.

In Table III a comparison is made of the total leucocytic counts and differential counts from the blood samples of the *tail* and the *aorta* of the same mouse.

It will be seen that the count from the aorta is decidedly lower than that taken from the tail and that the latter is from 1.4 to 5 times as much as the count from the aorta. In two cases it was respectively 11.8 and 8.7 times as much. The count from the aorta

TABLE III.

Comparison of Blood Samples taken from Tail and Aorta of Same Mouse.

Exp. No.	Diet.	W.B.C. Tail.	W.B.C. Aorta.	Ratio $\frac{A}{T}$	Differential Tail.				Differential Aorta.				
					Ly.	Mono.	Neutr.	Eos.	Ly.	Mono.	Neutr.	Eos.	
					SWISS MICE.								
48K.....	Alcohol.....	20,500	4,200	4·14	74	7	18	1	80	12	7	1	
121K.....	Tar Oil.....	9,430	3,500	2·69	85	3	10	2	20	2	2	1	(Only 25 cells counted).
122K.....	Sugar.....	13,000	3,600	3·8	64	8	24	3	70	6	21	3	
125K.....	Standard.....	10,400	—	—	75	2	19	4	78	8	11	3	
126K.....	Meat and Potatoes.	10,500	6,600	1·6	77	12	10	1	68	6	24	2	
129K.....	,, ,, .	28,400	9,200	3·0	69	13	16	2	66	16	16	2	
133K.....	Sugar.....	11,700	6,830	1·7	83	6	8	3	81	3	15	1	
134K.....	Standard.....	13,600	9,100	1·4	60	4	32	3	67	3	24	6	
137K.....	Sherman.....	—	2,800	—	91	2	3	4	47	—	2	1	(Only 50 cells counted.)
138K.....	Standard.....	—	1,700	—	77	6	11	6	45	2	2	1	,, ,,
143K.....	Sugar.....	9,100	3,200	2·8	73	13	13	1	29	5	11	5	,, ,,
150K.....	Coal Tar.....	16,400	1,400	11·8	90	—	8	2	45	2	3	—	,, ,,
151K.....	Fat.....	25,300	2,900	8·7	95	2	1	2	75	3	19	3	
185K	Fat.....	15,200	4,600	3·0	80	4	11	5	21	2	2	—	(Only 25 cells counted.)
186K.....	Fat.....	19,600	7,900	2·4	78	3	8	11	37	1	5	7	50 ,,
187K.....	Fat.....	15,800	4,100	3·8	80	5	11	4	42	4	3	1	,, ,,
192K.....	Salts 2.....	13,300	3,860	3·4	86	5	8	1	83	10	5	2	
193K.....	Salts 2.....	6,700	2,100	3·1	67	7	23	3	79	7	13	1	
194K.....	Salts 2.....	12,700	3,000	4·1	78	6	12	4	87	5	7	1	
196K.....	Alcohol.....	13,900	4,500	3·0	80	5	13	2	41	1	5	3	,, ,,
202K.....	Tar Oil.....	9,800	4,970	1·9	52	6	36	5	65	6	27	2	
203K.....	Tar Oil.....	12,100	2,200	5·5	69	8	14	9	71	3	20	6	
					ROCKEFELLER MICE.								
35K.....	Tar Oil.....	14,000	4,400	3·1	70	11	17	2	—	—	—	—	
130K.....	Meat and Potatoes.	6,700	3,500	1·9	75	11	6	8	72	13	11	4	
199K.....	Standard.....	11,300	2,530	4·4	77	4	19	2	39	4	4	3	(Only 50 cells counted.)
200K.....	Standard.....	16,100	3,700	4·3	60	5	18	7	43	2	2	3	,, ,,

was extremely low in some cases, e.g. 1,400, whereas it seldom exceeded 5,000. If the differential counts on the other hand are compared, it will be seen how remarkably closely these correspond. It would therefore appear that these low counts are not in any way associated with a drop in one type of leucocyte.

The various types of leucocytes were also considered in levels (see Tables IV, V. VI, and VII), and some of them were charted.

TABLE IV.

Lymphocytes %	2–3–4 months.		5–6–7–8 months.		1 year.		2 years.		Total.		Total R+S.	%
	R.	S.	R.	S.	R.	S.	R.	S.	R.	S.		
90	—	10	1	19	1	2	—	1	2	32	34	16·5
80	1	12	5	44	3	10	—	4	9	70	79	38·3
70	1	4	9	27	7	12	—	2	17	45	62	30·1
60	2	2	6	1	5	7	—	2	13	12	25	12·2
50	—	—	1	—	—	4	—	1	1	5	6	2·9

TABLE V.

Neutrophiles %	2–3–4 months.		5–6–7–8 months.		1 year.		2 years.		Total.		Total R+S.	%
	R.	S.	R.	S.	R.	S.	R.	S.	R.	S.		
5	—	7	—	12	2	2	—	1	2	22	24	11·5
10	—	7	3	27	3	4	—	2	6	40	46	22·1
15	2	5	7	23	3	7	—	1	12	36	48	23·07
20	1	5	5	18	2	4	—	3	8	30	38	18·2
25	1	2	2	9	3	6	—	1	6	18	24	11·5
30	—	2	3	1	3	6	—	—	6	9	15	7·2
35	—	—	1	4	1	4	—	—	2	8	10	4·8
40	—	—	1	—	—	1	—	2	—	3	3	1·4

TABLE VI.

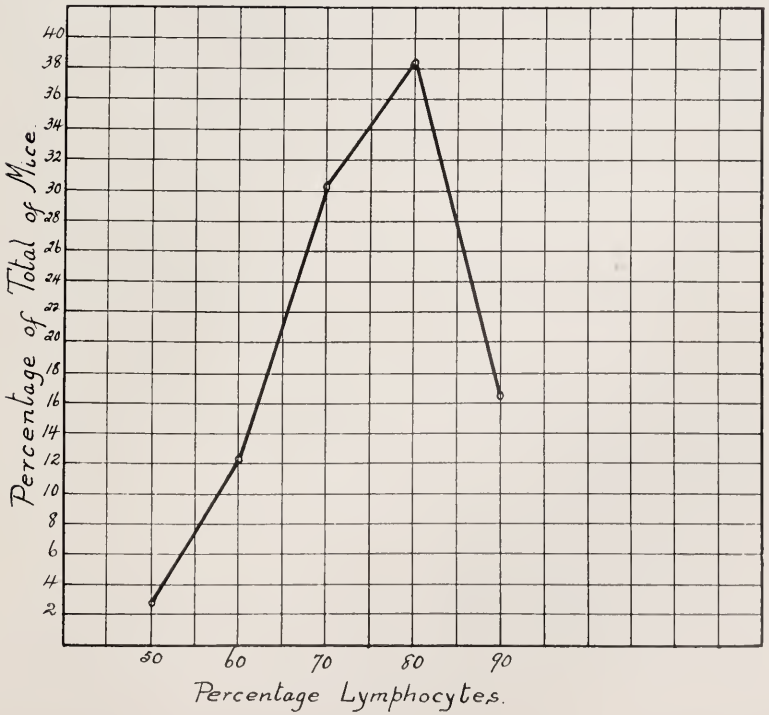
Eosinophiles %	2–3–4 months.		5–6–7–8 months.		1 year.		2 years.		Total.		Total R+S.
	R.	S.	R.	S.	R.	S.	R.	S.	R.	S.	
1	—	4	1	16	1	10	—	2	2	32	34
2	—	2	2	20	2	6	—	—	4	28	32
3	1	8	3	14	1	8	—	1	5	31	36
4	—	7	5	15	4	3	—	1	9	26	35
5	1	1	2	7	—	1	—	2	3	11	14
6	—	—	2	5	2	2	—	—	4	7	11
7	—	—	1	3	—	1	—	1	1	5	6
8	—	—	1	2	2	—	—	1	3	3	6
9	1	—	2	3	1	—	—	—	4	3	7
10	—	—	—	—	—	—	—	—	—	1	1
11	—	—	3	6	—	1	—	—	3	7	10
12	1	—	—	1	1	—	—	1	2	2	4

TABLE VII.

Monocytes. %	2–3–4 months.		5–6–7–8 months.		1 year.		2 years.		Total.		Total. R+S.
	R.	S.	R.	S.	R.	S.	R.	S.	R.	S.	
1	—	6	1	7	3	4	—	—	4	17	21
2	1	4	4	13	1	4	—	3	6	24	30
3	—	5	2	11	1	3	—	—	3	19	22
4	—	2	5	20	1	7	—	1	6	30	36
5	—	2	4	9	—	3	—	2	4	16	20
6	1	1	1	8	—	5	—	1	2	15	17
7	1	1	2	4	1	2	—	—	4	7	11
8	—	1	2	3	1	2	—	3	3	9	12
9	1	—	—	2	2	—	—	—	3	2	5
10	—	1	—	2	1	1	—	—	1	4	5
11	—	—	—	1	2	—	—	—	2	1	3
12	—	—	—	1	—	2	—	—	—	3	3
13	—	—	—	1	1	1	—	—	1	2	3
14	—	—	—	—	1	—	—	—	1	—	1

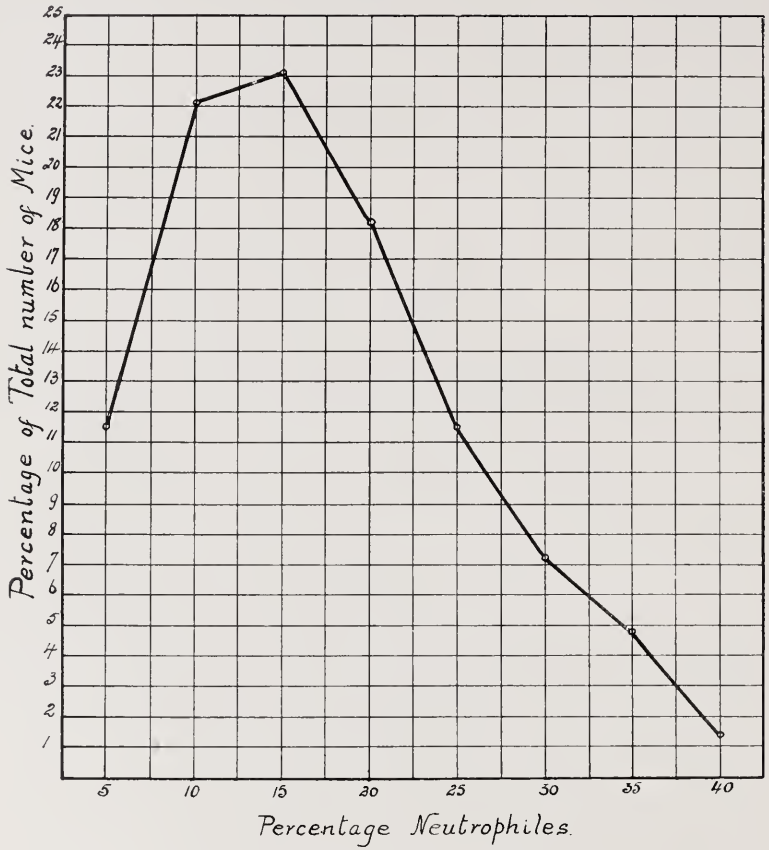
In case of the *lymphocytes* (Chart II), it will be seen that the greatest number of mice registered counts varying from 70-80 per cent.

CHART II.



In case of the *neutrophiles* (Chart III), the variation was from 10-20 per cent. with the greatest number at 15 per cent.

CHART III.



In Tables VI and VII the percentages *eosinophiles* and *monocytes* are also considered according to levels. It will be seen that the greatest number of mice showed 1-4 per cent. eosinophiles, and 1-8 per cent. monocytes.

Two mice registered 15 per cent. and 18 per cent. eosinophiles, and two 14 per cent. and 16 per cent. monocytes, respectively.

In the appendices there is a column of cells designated as "*degenerates.*" These represent in a number of the counts a small percentage of remains of cells in the fixed preparations which were impossible to classify. All that is seen is the remains of a nucleus in the form of a homogeneous chromatin mass lying wedged in between several erythrocytes. By virtue of this the outline assumes a very

irregular appearance. In a number of cases it gives one the impression that these cells were the remains of non-granular leucocytes, viz. monocytes and in others that of granulocytes. In spite of the presence of a small percentage of " degenerates " referred to in the columns of the appendices, it was remarkable how closely the differentials from fixed preparations coincided with those obtained by the supravital method.

In a few instances, therefore, the monocyte or neutrophile counts were probably slightly higher than represented by the figures given above.

It may be mentioned here that in some of the differential counts *Basophiles* were encountered and that these never exceeded 2 per cent.

Erythrocytic counts and grams of *haemoglobin* per 100 c.c. of blood are considered in Tables VIII and IX.

TABLE VIII.

Grams haemoglobin per 100 c.c. Blood.	2–3–4 months.		5–6–7–8 months.		1 year.		2 years.		Total.		Total. R+S.	%
	R.	S.	R.	S.	R.	S.	R.	S.	R.	S.		
25	—	—	—	3	—	—	—	1	—	4	4	1·5
24	—	—	—	2	—	—	—	—	—	2	2	0·75
23	—	—	1	6	—	—	—	—	1	6	7	2·6
22	2	4	7	8	—	3	—	—	9	15	24	9·1
21	1	3	6	15	—	5	—	2	7	25	32	12·0
20	4	11	2	32	4	3	—	1	10	47	57	21·5
19	1	4	9	22	5	9	—	1	15	36	51	19·2
18	—	2	5	12	5	8	—	3	10	25	35	13·2
17	—	3	4	8	3	3	—	2	7	16	23	8·6
16	—	1	1	1	8	4	—	2	9	8	17	6·4
15	—	1	—	2	4	2	—	4	4	9	13	4·9
14	—	1	—	—	—	—	—	—	—	1	1	0·38

TABLE IX.

Red Cells per Cubic mm.	2–3–4 months.		5–6–7–8 months.		1 year.		2 years.		Total.		Total R+S.	%
	R.	S.	R.	S.	R.	S.	R.	S.	R.	S.		
12	—	—	—	2	1	1	—	—	1	3	4	1·8
11	—	2	8	11	—	8	—	2	8	23	31	13·8
10	7	18	11	41	17	21	—	3	35	83	118	52·4
9	1	7	3	29	—	5	—	6	4	47	51	22·2
8	—	3	—	11	1	4	—	—	1	18	19	8·4
7	—	—	—	—	2	—	—	—	2	—	2	0·9

Likewise charts were prepared from the greatest number of mice represented in the different levels.

CHART IV.

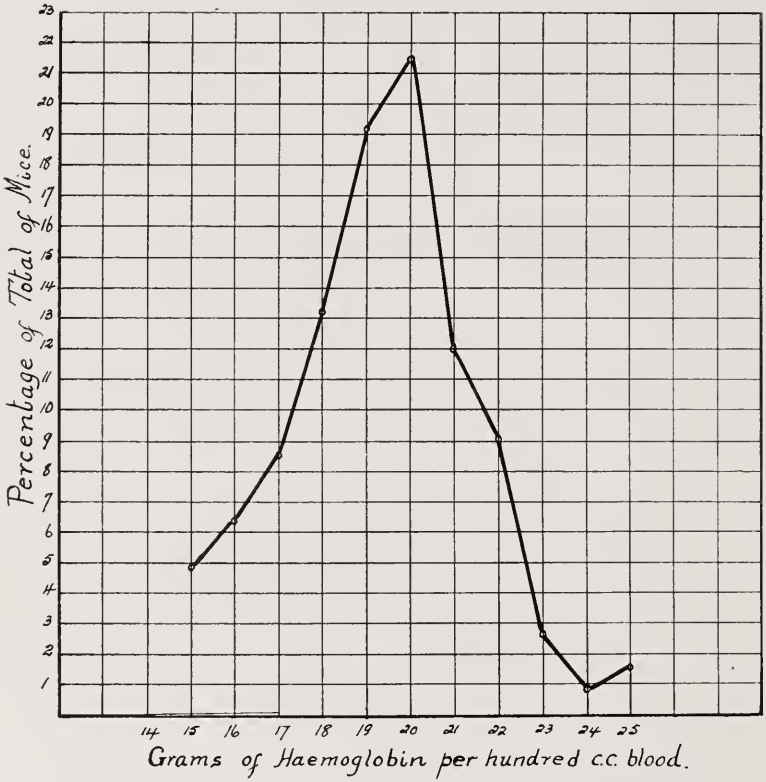
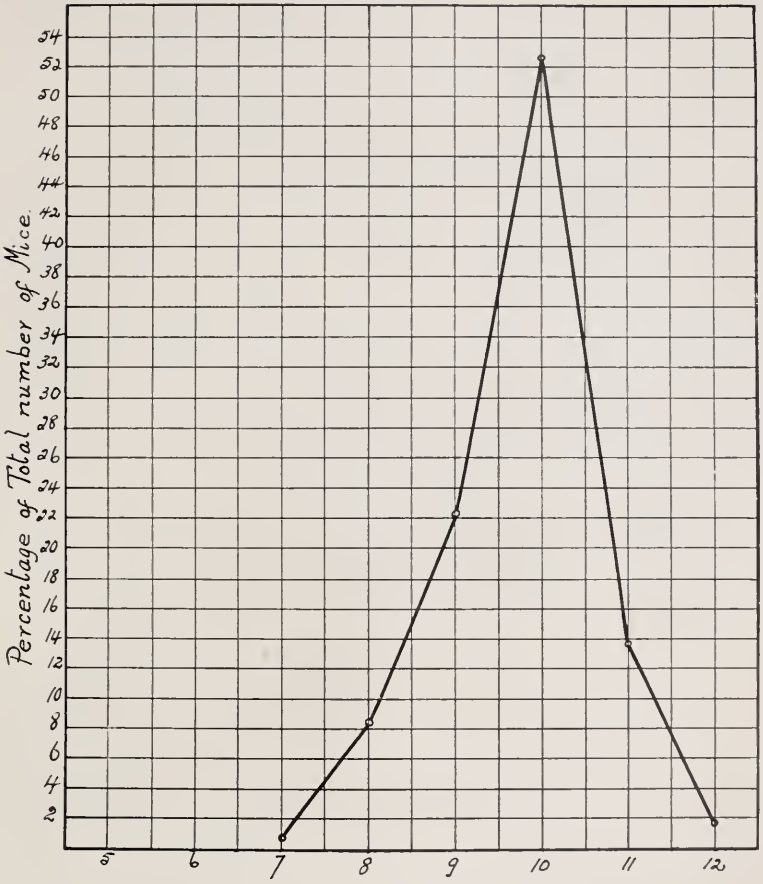

Percentage of Total of Mice.
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
14 15 16 17 18 19 20 21 22 23 24 25
Grams of Haemoglobin per hundred c.c. blood.

CHART V.



Red cells per c.mm. expressed in thousands.

In the case of *haemoglobin*, it will be seen from the chart that the highest levels were between 19-20 grams of haemoglobin per 100 c.c. of blood and from the figures obtained this would represent a fair average. Again it is interesting to note how closely the readings of the different samples of blood from the tail of the same mouse coincided.

The *erythrocytic count* (see Chart V), was fairly uniform and the greatest number of mice gave a reading of 10,000,000 red cells per cubic mm. Only a small number of mice registered counts as low as the 7,000,000 level and as high as 12,000,000.

In summarising the above findings, the following levels can be adopted for Rockefeller and Swiss mice:—

I. In respect of total *leucocyte* count great variations were found from 1,400-39,000. In case of blood from the *tail* 11,000-18,000 will represent a fair reading, whereas in case of blood from the *aorta* it is decidedly lower, viz. 2,000-4,000.

II. *The differential leucocytic counts* were more uniform and the following represent fair levels:—

Lymphocytes: 70-80 per cent.
Monocytes: 1-8 per cent.
Neutrophiles: 15-20 per cent.
Eosinophiles: 1-4 per cent.
Basophiles: -1 per cent.

III. In respect of the *erythrocytes* and grams of *haemoglobin* per 100 c.c. of blood, the following readings represent fair averages, viz:

10,000,000 red cells per cubic mm.
19-20 grams haemoglobin per 100 c.c. blood.

Discussion of the Literature.

How do the above data correspond with the findings of other investigators? Unfortunately the information concerning the blood of mice is very fragmentary, and wide discrepancies are found in the literature. Some of the most important references will be briefly considered here.

Hirschfeld (1919) gives the following figures for the blood of mice:—

Total leucocytic count: 7,800-12,000.
Differential count:
Lymphocytes: 60 per cent.
Monocytes: 12 per cent.
Neutrophiles: 25 per cent.
Eusinophiles: 3 per cent.
Erythrocytic count: 5.2 millions to 9 millions.
Haemoglobin: 85-100 per cent.

Remarkable, however, is the fact that Hirschfeld gives a very much higher leucocyte count (13,000-50,000) for mice affected with carcinoma. The Rockefeller and Swiss mice were all clinically healthy, and those with high levels autopsied revealed no tumour formations. It was, however, pointed out above that both these strains of mice were to a fairly large extent affected with mammary gland carcinoma. The question therefore arises whether the high levels established for total leucocytic count in this paper is to be attributed to mice in a kind of " precancerous " stage.

Koenigsveld and Kabierski (1915), however, also obtained a fairly high level (an average of 16,500) for total leucocytic count of mice, and they assert that the majority of mice show a tendency to exceed

the above level, a number of their mice registered counts over 35,000. Their other counts were as follows:—

Differential counts:
Lymphocytes: 60 per cent.
Monocytes: 10 per cent.
Neutrophiles: 27 per cent.
Eosinophiles: 3 per cent.

Red count: 8.2-14.8 millions and an average of 10,700,000. per cubic mm.

Haemoglobin: 90-105 per cent.

It is remarkable how their data correspond with those obtained by the author, especially in respect of total leucocytic and erythrocytic counts.

C. Kleinenberger (1927) gives some interesting data and he maintains that the absolute white count in the same animal changes when taken from the tail due to inflammatory changes and the addition of lymph. The percentage error may vary between 20-200 per cent. The white count from the tail is higher than from the aorta. No difference in the count exists whether the blood is taken during starvation or during feeding. He also found that the blood of mice coagulated rapidly. He gives the following interesting figures:—

	Tail.	*Aorta.*
Total leucocyte count:	7,500–13,200	569–3,960
Differential count:		
Large lymphocytes	9½ %	5–10½ %
Small lymphocytes	88½ %	65–86 %
Monocytes	½ %	–2½ %
Transitionals	2–5½ %	–1 %
Neutrophiles	8–45½ %	4–26 %
Eosinophiles	½–2½ %	–2 %
Basophiles	–½ %	–1 %
Red Count	7·9–11·7 million	7·4–10 million
Haemoglobin	94–106 %	99–128 %

If small and large lymphocytes, monocytes and transitionals, are considered together, it will be seen, except for the total leucocytic count from the tail, how closely these correspond with the figures obtained by the writer. Smith (1930) refers to certain discrepancies in the differential white count in infancy when compared by the supravital and fixed methods. He believes these to be accounted for by the unequal distribution and by the failure to identify all fragmented polymorph leucocytes in the fixed preparations, which gave erroneous impressions of lymphocytic preponderance. It was pointed out above, that with the method used by the writer, a very good distribution of cells was obtained in the smears prepared for differential counts. Moreover, the differential counts from a number of fixed preparations showed a small percentage of "degenerates," yet it was remarkable how closely the differentials from "fixed" and "supravital" preparations coincided.

Cheng (1929) in the study of the blood of rabbits asserts that wide variations in total leucocytic counts occurred amongst different members of the same litter kept under practically similar environmental conditions and given the same feed and care. If Table X is scrutinised, it will be seen that this was also the experience of the writer.

TABLE X

ROCKEFELLER MICE—SAME LITTER.

Diet.	Expt.	Age.	Time.	Tail.	Date Bled.	R.B.C.	Hg.	W.B.C.	Ly.S.Ly.	Mono.	Neut.	Eos.	Degen.
Complete....	157K.....	3 months	11.30....	T_1....	June 4....	9·04	19·9	16,800	58	6	24	12	1
	157K.....		11.33....	T_2....			19·6	16,200	—	—	—	—	—
	158K.....	3 months	11.40....	T_1....	June 4....	9·56	19·6	15,800	61	9	21	9	—
	158K.....		11.40....	T_2....			19·4	14,100	—	—	—	—	—
	159K.....	3 months	2.40....	T_1....	June 4....	10·46	22·3	9,700	74	7	14	5	1
	159K.....		2.43....	T_2....			22·3	7,700	—	—	—	—	—
	160K.....	3 months	2.55....	T_1....	June 4....	10·41	20·9	11,400	80	2	15	3	3
	160K.....		2.58....	T_2....			20·3	9,700	—	—	—	—	—
					ROCKEFELLER MICE—SAME LITTER.								
Complete....	161K.....	12 months	9.35....	T_1....	June 5....	9·72	—	13,100	—	—	—	—	—
	161K.....		9.38....	T_2....			—	10,100	—	—	—	—	—
	162K.....	12 months	9.45....	T_1....	June 5....	10·32	—	13,300	—	—	—	—	—
	162K.....		9.48....	T_2....			—	7,400	—	—	—	—	—
	163K.....	12 months	10.55....	T_1....	June 5....	9·52	—	9,400	—	—	—	—	—
	163K.....		10.08....	T_2....			—	9,000	—	—	—	—	—
	164K.....	12 months	11.05....	T_1....	June 5....	9·70	—	13,700	—	—	—	—	—
	164K.....			T_2....			—	10,400	—	—	—	—	—

According to Cheng each individual rabbit tended to have a characteristic type of total leucocytic count during the first one and a half years, and for this reason he recognised three types, viz. high, medium and low.

The writer could not confirm Cheng's conclusions that a different position of the animal during bleeding causes a corresponding change in total leucocytic counts from the heart and the ear blood. Cheng believes that the polymorphs and lymphocytes play distinct parts so that the leucopenia at the " abdomen up " position is caused by a decrease of the lymphocytes, etc.

In blood studies (*a*) on splenectomised, etc., ruminants, De Kock and Quinlan (1926), and (*b*) on equines, De Kock (1923), assume the existence of " individual " levels of total leucocytic count. In these studies it was therefore deemed essential in each particular animal to study the blood first during health, and then during disease, or after splenectomy. In no instance was an " average " for a particular species of animal accepted or adopted.

Morphological Considerations of the Leucocytes of Mice.

These were studied (*a*) in fixed preparations stained by the May-Grunwald-Giemsa method, (*b*) in supravitally stained preparations, and (*c*) in tissue cultures according to the methods described above.

In this study the following classification of the leucocytes of the blood of mice were adopted: lymphocytes, monocytes, neutrophiles, eosinophiles, and basophiles.

No attempt at further sub-division was made, in spite of the fact that Kleinberger and others speak of small and large lymphocytes, and that Sabin and her co-workers sub-divide the macrophages of the blood into monocytes and clasmatocytes. When these leucocytes are studied in cultures and their mode of progression cinematographically followed, it will be seen that only three groups of leucocytes can be identified, viz. the *polymorphs* (mainly the neutrophiles), the *macrophages* (i.e. the monocytes and the clasmatocytes of Sabin), and *lymphocytes*. In spite of the fact that monocytes and clasmatocytes may be differentiated by certain features produced by the supravital stain, the evidence produced by the film of living cultures seem to indicate that these types, i.e. monocytes and clasmatocytes are most likely one and the same cell in different stages of physiological activity. When stained supravitally these macrophages mainly showed neutral red stained granules resembling those of the so-called clasmatocyte of Sabin, yet in the fixed preparations they all resembled that type of cell classified by us as monocyte.

In tissue cultures medium sized cells after 6 hours are found at the periphery of the medium and scattered amongst them, but less frequent are those cells identified as macrophagi. They are distinctly larger, usually have a constricted appearance about the centre. When these preparations are fixed and stained with Giemsa, it will be seen that the medium shaped cells at the periphery were mainly neutrophiles, whereas the larger macrophagi or monocytes are more scattered and amongst them a few lymphocytes could be identi-

fied. In these culture preparations, apart from the supravital stain, it was never possible to identify two types of large macrophage, viz. monocyte and clasmatocyte.

It was pointed out above that it was not possible to evolve a method which gave a regular migration of leucocytes in every instance. The presence of large numbers of *Thrombocytes* in the blood of mice seems to play an important part in this respect, and the dense white film formed around the explant most probably prevents the migration of leucocytes. These films representing dense masses of thrombocytes may accumulate at the periphery of the explant almost immediately after it was made. Various unsuccessful attempts were made to suppress this by trying various combinations of media and changes in the technique.

The *neutrophiles* of the blood of mice appear almost transparent although with prolonged staining it is possible to demonstrate fine and very scanty neutrophile granules. The nuclei are much segmented, and supravitally these cells can easily be identified by their movement, glassy appearance and their fine granules which are continuously streaming in the direction the cell is moving. As a result of the gradual progression of the cell, it presents various shapes, usually elongated.

The *lymphocyte* is usually of the medium size type. Their nuclei fill almost the greater portion of the cell, only a small seam can be identified at one pole. In the small lymphocyte often only a trace of cytoplasm can be spotted. In these cells it would appear as if the nucleus fills the whole cell. Seldom lymphocytes with a large amount of cytoplasm are encountered in the blood of mice. This type of cell is not uncommon in the blood of sheep, and De Kock (1928) states that he frequently had great difficulty in discriminating between these and monocytes. The blood of mice in this respect offered very little difficulty when making differential counts in fixed preparations.

Supravitally the mitochondriae stain prominently, and often a few small neutral red granules were also found present.

The *monocytes* are the largest cells, and in stained preparations usually showed the characteristic bean or kidney-shaped nucleus and a great deal of cytoplasm of a greyish blue colour. In supravitally stained slides these cells are usually round with the typical neutral red stained granules.

The *eosinophile* of the blood of mice is fairly characteristic and easily identified in stained preparations by its eosinophilic granules, and non-segmented ring-shaped nucleus, which is sometimes S-shaped, with the one limb of the S closed. The granules are frequently situated in the centre of the cell, but in some, granules may be found between the periphery of the cell and the nucleus, as well as in the centre of a ring-shaped nucleus. At the periphery in these cases the granules are disseminated through an almost transparent cytoplasm. The granules are not always uniform, on account of differences in size and staining intensity. In supravitally prepared slides these cells are usually round and the granules assume a very faint lemon yellow colour. In the blood of mouse 84K mitochondriae were found amongst the granules, although this is a distinctly rare occurrence.

Conclusions.

1. By eliminating as far as it was possible various factors, e.g. daily rhythm, mechanical interferences, disease, age, sex, race, diet, environment, which may have operated in this study of the blood of mice, it was not possible to explain the cause of the variations obtained, especially of total leucocytic count.

2. It was considered advisable to express total leucocytic count of the mouse rather in levels than in averages on account of the great variation from 4,000-39,000. The greatest percentage of mice, however, varied between 11,000-18,000 leucocytes per cubic mm.

3. The total leucocytic count from the *tail* was about 1.4 to 5 times as much as that from the *aorta*, whereas the differential counts closely coincided.

4. 19-20 grams of haemoglobin per 100 c.c. of blood and 10 millions erythrocytes per c.mm. represented fair levels for the blood of Rockefeller and Swiss mice.

5. For differential counts the following represent fair levels:—

Lymphocytes: 70-80 per cent.
Monocytes: 1-8 per cent.
Neutrophiles: 15-20 per cent.
Eosinophiles: 1-4 per cent.
Basophiles: 1 per cent.

6. Morphological considerations are given of the various leucocytes of the blood of mice in fixed and supravital preparations, and in cultures.

Acknowledgements.

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APPENDIX I.—*Blood Counts for two strains of Mice at various ages and times of bleeding, arranged according to different diets.*

SWISS MICE: 2–3–4 MONTHS.

Diet.	Experimental No.	Age.	Time.	Aorta Tail.	Date Bled.	Red Blood Count.	Haemoglobin.	White Blood Count.	Lymphocytes.	Monocytes.	Neutrophiles.	Eosinophiles.	Degenerated Cells.
					1930.		Gm. per 100 cc.						
Standard	12 K.	4 mo.	2.40 p.m.	T_1	Apr. 3	10·08	20·00	25,400	77	5	17	—	—
Complete	21 K.	2 mo.	2.40 p.m.	T_1	Apr. 7	9·14	21·1	13,100	79	1	22	3	6
	62 K.	3 mo.	11.00 a.m.	T_1	Apr. 24	10·57	18·7	11,900	79	2	19	—	1
	62 K.	—	11.04 a.m.	T_2	Apr. 24	—	—	11,500	—	—	—	—	—
Sherman	14 K.	3 mo.	11.30 a.m.	T_1	Apr. 4	9·66	20·35	25,000	90	1	5	4	—
	64 K.	3 mo.	2.35 p.m.	T_1	Apr. 24	8·99	15·82	8,200	—	—	—	—	—
	64 K.	—	2.39 p.m.	T_2	Apr. 24	—	15·28	7,070	—	—	—	—	—
Sherman + Garlic	17 K.	2 mo.	10.40 a.m.	T_1	Apr. 5	8·47	20·00	9,400	61	3	32	4	—
	65 K.	3 mo.	11.35 a.m.	T_1	Apr. 25	10·01	17·66	10,000	84	1	12	3	6
	65 K.	—	11.38 a.m.	T_2	Apr. 25	—	—	10,300	—	—	—	—	—
Sherman + Sugar	19 K.	2 mo. +	11.40 a.m.	T_1	Apr. 7	9·95	21·5	13,900	80	3	13	4	—
	67 K.	3 mo. +	2.55 p.m.	T_1	Apr. 25	10·43	19·00	19,500	88	2	6	4	—
	133 K.	4 mo. +	11.30 a.m.	T_1	May 26	10·46	—	11,700	83	6	8	3	—
	133 K.	—	11.40 a.m.	A_1	May 26	8·55	—	6,830	81	3	15	1	1
Sherman + Tar Oil	18 K.	2 mo. +	11.10 a.m.	T_1	Apr. 7	9·44	19·65	19,200	77	1	18	4	—
	66 K.	3 mo.	1.45 p.m.	T_1	Apr. 25	10·65	19·3	10,400	73	4	21	2	—
	66 K.	—	1.48 p.m.	T_2	Apr. 25	—	19·00	8,000	—	—	—	—	—
Sherman + Meat and Potatoes	20 K.	2 mo.	2.15 p.m.	T_1	Apr. 7	10·19	17·27	6,700	38	—	12	—	—
	76 K.	3 mo.	2.48 p.m.	T_1	Apr. 29	8·82	16·5	7,900	70	3	24	3	—
	141 K.	4 mo.	2.05 p.m.	T_1	May 27	8·16	14·0	9,530	70	2	24	3	5
	141 K.	—	2.08 p.m.	T_2	May 27	—	—	7,600	—	—	—	—	—
Sherman + Fat	13 K.	3 mo.	11.05 a.m.	T_1	Apr. 4	10·00	22·2	23,300	96	—	3	1	—
	68 K.	3 mo.	11.00 a.m.	T_1	Apr. 26	9·69	20·35	24,100	86	1	10	3	—
	68 K.	—	11.03 a.m.	T_2	Apr. 26	—	—	21,900	—	—	—	—	—
	151 K.	5 mo.	11.05 a.m.	T_1	May 29	9·43	—	25,300	—	—	—	—	—
	—	—	—	A_1	May 29	9·39	—	2,900	—	—	—	—	—
Sherman + Arsenic	33 K.	3 mo. +	11.40 a.m.	T_1	Apr. 10	7·95	17·56	14,300	80	8	11	1	4
	77 K.	3 mo. +	1.25 p.m.	T_1	Apr. 30	9·67	19·8	9,700	79	1	21	4	—
	77 K.	—	1.28 p.m.	T_2	Apr. 30	—	—	6,500	—	—	—	—	—
Sherman + Salts Experiment 2	15 K.	3½ mo.	2.20 p.m.	T_1	Apr. 4	9·81	20·00	19,300	86	—	14	—	—
	69 K.	4 mo. +	1.50 p.m.	T_1	Apr. 28	10·58	20·3	16,600	82	7	10	1	—
Sherman + Raw Meat	10 K.	4 mo.	10.55 a.m.	T_1	Apr. 3	10·07	21·32	17,400	80	5	14	1	—
Sherman + Aqueous Tar	16 K.	3 mo.	2.40 p.m.	T_1	Apr. 4	15·08	21·3	33,900	95	—	3	2	—
	Ether												
	92 K.	4 mo.	2.45 p.m.	T_1	May 7	10·60	16·8	12,300	85	3	9	3	—
Sherman + Alcohol	54 K.	3 mo.	3.10 p.m.	T_1	Apr. 21	10·08	20·1	9,800	92	—	4	4	—
	54 K.	—	3.13 p.m.	T_2	Apr. 21	—	—	13,200	—	—	—	—	—
	81 K.	3 mo.	2.15 p.m.	T_1	May 1	10·01	21·5	17,800	88	4	5	3	5
	81 K.	—	2.20 p.m.	T_2	May 1	—	—	16,800	—	—	—	—	—
	148 K.	4 mo. +	2.30 p.m.	T_1	May 29	10·38	20·9	17,200	86	2	7	5	5
	148 K.	—	2.33 p.m.	T_2	May 29	—	20·7	16,400	—	—	—	—	—
Complete Greenhouse	22 K.	4 mo.	9.40 a.m.	T_1	Apr. 8	9·62	21·95	13,400	60	10	30	—	3

Abbreviations.—T_1 = First sample of blood from tail. T_2 = Second sample of blood from tail. Mo. = Months. A = Aorta.

SWISS MICE: 5–6–7 MONTHS.

Diet.	Experimental No.	Age.	Time.	Aorta Tail.	Date Bled.	Red Blood Count.	Haemoglobin.	White Blood Count.	Lymphocytes.	Monocytes.	Neutrophiles.	Eosinophiles.	Degenerated Cells.	Doubtful.
					1930.		Gm. per 100 cc.							
Standard	2 K.	6 mo.	10.50 a.m.	T_1	Apr. 1	9·96	20·7	12,300	70	—	25	4	—	1
	63 K.	5 mo. +	11.45 a.m.	T_1	Apr. 24	10·46	15·3	16,600	81	4	11	4	—	—
	63 K.	—	11.48 a.m.	T_2	Apr. 24	—	17·5	19,800	—	—	—	—	—	—
	75 K.	7 mo.	2.03 p.m.	T_1	Apr. 29	10·00	21·7	14,300	75	2	14	9	—	—
	25 K.	6 mo.	11.45 a.m.	T_1	Apr. 8	9·36	20·3	8,300	87	—	11	—	2	—
	111 K.	8 mo.	1.50 p.m.	T_1	May 16	10·81	20·3	15,100	75	4	15	6	—	—
	111 K.	—	1.53 p.m.	T_2	May 16	—	17·9	13,000	—	—	—	—	—	—
	138 K.	8 mo.	9.25 a.m.	T_2	May 27	—	—	—	77	6	11	6	9	—
	*138 K.†	—	9.40 a.m.	A_1	May 27	9·86	—	1,250	45	2	1	2	—	—
	169 K.	7 mo.	10.20 a.m.	T_1	June 10	—	—	20,300	80	8	9	2	15	—
	170 K.	7 mo.	10.30 a.m.	T_1	June 10	9·75	18·7	22,000	83	9	3	5	—	—
	171 K.	7 mo.	10.50 a.m.	T_1	June 10	11·27	17·8	15,400	80	4	14	2	3	—
	171 K.	—	—	T_2	June 10	—	15·5	10,900	—	—	—	—	—	—
	172 K.	7 mo.	1.50 p.m.	T_1	June 10	12·39	19·4	8,400	83	6	8	3	—	—
	172 K.	—	—	T_2	June 10	—	21·1	20,200	—	—	—	—	—	—
Complete	3 K.	6 mo.	2.10 p.m.	T_1	Apr. 1	8·26	16·5	21,600	80	4	14	2	—	—
	78 K.	7 mo.	2.04 p.m.	T_1	Apr. 30	10·98	19·6	15,800	71	3	23	3	—	—
	23 K.	6 mo.	10.15 a.m.	T_1	Apr. 8	9·96	19·3	21,700	93	—	7	—	1	—
	173 K.	6 mo.	11.15 a.m.	T_1	June 11	12·2	24·7	14,000	72	4	23	1	—	—
	173 K.	—	11.18 a.m.	T_2	June 11	—	24·5	8,800	—	—	—	—	—	—
	174 K.	6 mo.	11.25 a.m.	T_1	June 11	10·4	22·9	12,300	75	2	22	1	6	—
	174 K.	—	11.28 a.m.	T_2	June 11	—	22·8	11,000	—	—	—	—	—	—
	175 K.	6 mo.	2.15 p.m.	T_1	June 11	9·59	22·1	10,400	62	7	19	11	4	—
	176 K.	6 mo.	2.55 p.m.	T_1	June 11	9·22	17·5	13,900	72	3	19	6	—	—
Sherman	1 K.	5 mo.	10.10 a.m.	T_1	Apr. 1	10·36	22·8	18,300	42	2	53	1	—	2
	79 K.	6 mo.	2.33 p.m.	T_1	Apr. 30	9·13	21·15	9,700	83	—	15	2	4	—
	79 K.	—	2.37 p.m.	T_2	Apr. 30	—	—	6,200	—	—	—	—	—	—
	24 K.	5 mo. +	11.15 a.m.	T_2	Apr. 8	9·08	19·49	6,350	94	2	4	—	—	—
	137 K.	7 mo.	9.15 a.m.	T_3	May 27	—	—	—	89	2	3	4	—	—
	†137 K.	—	9.30 a.m.	A_1	May 27	10·46	—	2,800	47	—	2	1	2	2
	177 K.	7 mo.	9.25 a.m.	T_1	June 12	10·16	18·7	12,900	70	5	21	2	2	8
	177 K.	7 mo.	9.28 a.m.	T_2	June 12	—	18·8	12,400	—	—	—	—	—	—
	178 K.	7 mo.	9.40 a.m.	T_1	June 12	8·44	18·5	10,200	69	4	24	3	3	—
	178 K.	—	—	T_2	June 12	—	18·0	7,700	—	—	—	—	—	—
	179 K.	7 mo.	10.50 a.m.	T_1	June 12	10·18	—	14,700	79	2	15	4	—	—
	180 K.	7 mo.	11.10 a.m.	T_1	June 12	9·06	19·0	10,800	78	5	12	5	1	—
	180 K.	—	—	T_2	June 12	—	18·5	7,800	—	—	—	—	—	—

Abbreviations.—T_1 = First sample of blood from tail. T_2 = Second sample of blood from tail. Mo. = Months. A = Aorta.

* Killed May 27th. † Only 50 cells counted.

Diet.	Experimental No.	Age.	Time.	Aorta Tail.	Date Bled.	Red Blood Count.	Haemoglobin.	White Blood Count.	Lymphocytes.	Monocytes.	Neutrophiles.	Eosinophiles.	Degenerated Cells.	Doubtful.
					1930.		Gm. per 100 cc.							
Sherman + Garlic.....	4 K.	7 mo.	3.00 p.m.	T_1	Apr. 1	10·48	21·24	15,200	77	4	13	6	—	—
	80 K.	9 mo.	2.00 p.m.	T_1	May 1	—	—	—	74	3	21	2	—	—
	26 K.	7 mo.	2.30 p.m.	T_2	Apr. 8	—	—	—	90	4	5	1	—	—
	181 K.	7 mo.	11.20 a.m.	T_1	June 16	9·91	20·3	15,200	84	4	11	1	2	—
	181 K.	—	—	T_2	June 16	—	19·9	13,600	—	—	—	—	—	—
	182 K.	7 mo.	11.35 a.m.	T_1	June 16	9·77	20·5	4,200	26	7	16	1	—	—
	182 K.	—	—	T_2	June 16	—	20·0	5,500	—	—	—	—	—	—
	183 K.	7 mo.	1.45 p.m.	T_1	June 16	10·35	18·7	16,100	85	4	8	3	7	—
	183 K.	—	1.48 p.m.	T_2	June 16	—	19·1	14,800	—	—	—	—	—	—
	184 K.	7 mo.	2.00 p.m.	T_1	June 16	9·82	20·5	7,200	75	2	16	7	2	—
	184 K.	—	2.03 p.m.	T_2	June 16	—	20·9	6,600	—	—	—	—	—	—
Sherman + Sugar.....	5 K.	6½ mo.	11.05 a.m.	T_1	Apr. 2	9·23	14·17	11,400	75	12	12	1	—	—
	85 K.	7 mo.	2.15 p.m.	T_1	May 5	10·17	19·1	9,900	73	2	14	11	4	—
	143 K.	8 mo.	9.30 a.m.	T_3	May 28	8·73	—	9,100	73	13	4	—	—	—
	*143 K.†	—	9.40 a.m.	A_1	May 28	7·96	—	3,200	29	5	11	5	—	—
	188 K.	7 mo.	11.10 a.m.	T_1	June 18	10·18	22·5	12,700	76	3	17	4	—	—
	189 K.	7 mo.	11.23 a.m.	T_1	June 18	9·01	23·4	19,500	49	5	31	15	—	—
	189 K.	—	11.26 a.m.	T_2	June 18	—	20·3	13,300	—	—	—	—	—	—
	190 K.	7 mo.	2.00 p.m.	T_1	June 18	10·49	23·9	16,000	67	8	14	11	2	—
	190 K.	7 mo.	2.03 p.m.	T_2	June 18	—	21·5	14,700	—	—	—	—	—	—
	191 K.	7 mo.	2.20 p.m.	T_1	June 18	8·91	19·8	18,900	73	4	21	2	—	—
	191 K.	—	2.23 p.m.	T_2	June 18	—	19·8	14,800	—	—	—	—	—	—
Sherman + Tar Oil.....	6 K.	5 mo.	11.20 a.m.	T_1	Apr. 2	9·58	20·16	15,600	72	6	18	4	—	—
	82 K.	6 mo. +	2.50 p.m.	T_1	May 1	9·85	16·5	6,300	75	1	20	4	2	—
	82 K.	—	—	T_2	May 1	—	—	4,700	—	—	—	—	—	—
	28 K.	5 mo. +	11.30 a.m.	T_2	Apr. 9	—	—	—	—	—	—	—	—	—
	‡201 K.	8 mo.	9.55 a.m.	T_1	June 24	8·35	21·7	9,800	79	5	13	3	9	—
	202 K.	8 mo.	10.05 a.m.	T_1	June 24	8·61	22·3	9,800	52	6	36	5	5	—
	‡202 K.	—	10.15 a.m.	A_1	June 24	8·31	—	4,970	65	6	27	2	2	—
	203 K.	8 mo.	11.15 a.m.	T_1	June 24	8·67	19·3	12,100	69	8	14	9	—	—
	‡203 K.	—	11.25 a.m.	A_1	June 24	8·30	—	2,200	71	3	20	6	6	—
Sherman + Meat and Potatoes	7 K.	5 mo.	2.10 p.m.	T_1	Apr. 2	9·09	25·3	19,500	89	—	10	1	—	—
	84 K.	6 mo.	11.50 a.m.	T_1	May 5	10·37	19·6	12,500	71	1	18	8	7	—
	84 K.	6 mo.	11.53 a.m.	T_2	May 5	—	17·8	11,100	—	—	—	—	—	—
	30 K.	5 mo.	12.00 noon	T_2	Apr. 9	—	18·08	13,800	—	—	—	—	—	—
	144 K.	7 mo.	9.35 a.m.	T_1	May 28	—	—	—	(very few leucocytes.)			—	—	—
	*144 K.	—	9.45 a.m.	A_1	May 28	—	—	—	—	—	—	—	—	—
	§204 K.	8 mo.	11.30 a.m.	T_1	June 25	9·64	21·71	12,700	83	2	14	1	2	—
	204 K.	8 mo.	11.30 a.m.	T_2	June 25	—	20·35	11,500	—	—	—	—	—	—
	205 K.	8 mo.	2.35 p.m.	T_1	June 25	8·43	16·5	4,800	67	3	26	4	2	—
	205 K.	8 mo.	2.38 p.m.	T_2	June 25	—	16·2	3,800	—	—	—	—	—	—
	206 K.	8 mo.	2.55 p.m.	T_1	June 28	9·05	20·5	4,500	73	6	19	2	3	—
	206 K.	8 mo.	2.58 p.m.	T_2	June 28	—	19·1	3,300	—	—	—	—	—	—

Abbreviations.—T_1 = First sample of blood from tail. T_2 = Second sample of blood from tail. Mo. = Months. A = Aorta.
* Killed May 28th. † Only 50 cells counted. ‡ Killed June 24th. § Killed by other mice June 25th.

SWISS MICE: 5-6-7 MONTHS—(*continued*).

Diet.	Experimental No.	Age.	Time.	Aorta Tail.	Date Bled.	Red Blood Count.	Haemoglobin.	White Blood Count.	Lymphocytes.	Monocytes.	Neutrophiles.	Eosinophiles.	Degenerated Cells.	Doubtful.
					1930.		Gm. per 100 cc.							
Sherman + Fat.....	8 K.	6 mo.	2.30 p.m.	T_1	Apr. 2	10·19	17·99	14,900	77	1	22	12	3	
	86 K.	7 mo.	2.45 p.m.	T_1	May 5	10·62	17·13	15,800	74	4	10			
	86 K.		2.48 p.m.	T_2	May 5	—	—	12,900						
	27 K.	7 mo.	11.15 a.m.	T_1	Apr. 9	10·92	23·6	10,900	90	3	3	4	6	
	151 K.	5 mo.	11.05 p.m.	T_1	May 29	9·43	—	25,300	95	2	1	2	3	
	151 K.		11.15 a.m.	T_2	May 29	9·39	—	2,900	75	3	19	3	2	
	*185 K.	7 mo.	9.40 a.m.	A_1	May 29	9·69	18·39	15,200	80	4	11	2		
	*185 K.			A_1	June 17	8·40	—	4,600	21		2			
	*186 K.	7 mo.	10.10 a.m.	T_1	June 17	9·64	19·4	19,600	78	3	8	1		
	*186 K.		10.20 a.m.	A_1	June 17	9·60	—	7,900	37	1	5	1		
	*187 K.	7 mo.	10.25 a.m.	T_1	June 17	10·11	20·9	15,800	80	5	11	4	24	
	†187 K.		—	A_1	June 17	9·13	—	4,100	42	4	3	1	8	
Sherman + Arsenic.....	9 K.	6 mo.	10.40 a.m.	T_1	Apr. 3	9·48	20·36	17,400	78	2	18	2		
	83 K.	8 mo.	11.30 a.m.	T_1	May 5	11·16	19·9	12,600	55		34	11		
	83 K.		11.33 a.m.	T_2	May 5	—	19·9	9,500						
	207 K.	5 mo.	9.25 a.m.	T_1	June 26	9·23	20·5	14,900	87	4	7	2	3	
	207 K.		9.28 a.m.	T_2	June 26	—	19·3	11,200						
	208 K.	5 mo.	9.45 a.m.	T_1	June 26	9·77	20·7	13,500	81	9	6	4	1	
	208 K.		9.48 a.m.	T_2	June 26	—	20·3	11,600						
	209 K.	5 mo.	10.25 a.m.	T_1	June 26	8·91	22·5	14,700	68	4	24	4	2	
	209 K.		10.28 a.m.	T_2	June 26	—	21·1	12,900						
	210 K.	5 mo.	10.35 a.m.	T_1	June 26	10·72	20·5	13,300	81	4	11	4		
	210 K.		10.38 a.m.	T_2	June 26	—	17·9	14,100						
Sherman + Salts, Experiment No. 2	11 K.	5 mo.	2.15 p.m.	T_1	Apr. 3	9·42	20·19	14,900	78	4	17	1		
	88 K.	7 mo.	1.50 p.m.	T_1	May 6	9·54	17·24	13,500	70	4	25	1		
	88 K.		—	T_2	May 6	—	17·8	11,600	86	5	8	1	5	
	192 K.	7 mo.	10.05 a.m.	T_1	June 19	8·55	20·72	13,300	83	5	5	2		
	‡192 K.	7 mo.	—	A_1	June 19	—	—	3,400	67	10	23	3		
	193 K.	7 mo.	10.20 a.m.	T_1	June 19	8·67	18·8	6,700	79	1	13	1	2	
	‡193 K.	7 mo.	10.30 a.m.	A_1	June 19	8·65	—	2,100	79	1	9	1	2	
	†193 K.	7 mo	10.25 a.m.	Vein$_4$	June 19	9·99	19·6	3,800	40	1	12	1		
	194 K.	7 mo.	11.45 a.m.	T_1	June 19	8·70	19·8	12,700	78	6	14	4		
	‡194 K.		11.55 a.m.	A_1	June 19	8·00	—	3,000	74	11	14	1		

Abbreviations.—T_1 = First sample of blood from tail. T_2 = Second sample of blood from tail. Mo. = Months. A = Aorta.

* Killed June 17th. † Only 50 cells counted. ‡ Killed June 19th.

SWISS MICE: 5-6-7 MONTHS—(*continued*).

Diet.	Experimental No.	Age.	Time.	Aorta Tail.	Date Bled.	Red Blood Count.	Haemoglobin.	White Blood Count.	Lymphocytes.	Monocytes.	Neutrophiles.	Eosinophiles.	Degenerated Cells.	Doubtful.
					1930.		Gm. per 100 cc.							
Sherman + Raw Meat.....	70 K.	5 mo.	2.35 p.m.	T_1	Apr. 28	10·01	20·5	21,600	85	—	12	5	—	—
	110 K.	7 mo.	1.53 p.m.	T_1	May 15	8·95	16·6	14,000	51	4	38	8	—	—
	110 K.	—	1.56 p.m.	T_2	May 15	—	16·5	12,200	—	—	—	—	—	—
	112 K.	5 mo. +	2.20 p.m.	T_1	May 16	9·32	19·1	8,500	75	3	19	3	3	—
	211 K.	5 mo. +	1.50 p.m.	T_1	June 26	9·34	21·3	17,900	76	1	20	3	—	—
	211 K.	5 mo. +	1.53 p.m.	T_2	June 26	—	20·9	14,300	—	—	—	—	—	—
	212 K.	5 mo. +	2.05 p.m.	T_1	June 26	9·23	19·6	22,200	70	3	18	9	4	—
	212 K.	5 mo. +	2.05 p.m.	T_2	June 26	—	19·9	15,200	—	—	—	—	—	—
	213 K.	7 mo.	11.15 a.m.	T_1	June 27	10·69	22·3	20,000	78	5	14	2	—	—
	213 K.	7 mo.	11.18 a.m.	T_2	June 27	—	22·3	20,200	—	—	—	—	—	—
	214 K.	7 mo.	11.25 a.m.	T_1	June 27	9·72	21·1	18,000	81	4	12	3	—	—
	214 K.	7 mo.	11.28 a.m.	T_2	June 27	—	21·3	15,800	—	—	—	—	—	—
Sherman + Aqueous Tar...	150 K.	6 mo.	11.00 a.m.	T_1	May 29	10·07	—	16,400	90	—	8	2	3	—
	*150 K.†	6 mo.	11.10 a.m.	A_1	May 29	9·47	—	1,400	45	2	3	—	1	—
Sherman + Alcohol.....	53 K.	6 mo.	2.20 p.m.	T_1	Apr. 21	10·84	20·5	5,900	89	—	15	1	—	—
	53 K.	—	2.23 p.m.	T_2	Apr. 21	—	20·0	7,800	—	—	—	—	—	—
	91 K.	6 mo.	2.15 p.m.	T_1	May 7	10·37	18·5	18,600	79	6	12	2	—	—
	153 K.	7 mo.	2.30 p.m.	T_1	June 2	10·20	20·9	12,800	81	5	7	4	3	3
	153 K.	7 mo.	2.33 p.m.	T_2	June 2	—	—	17,000	—	—	—	—	—	—
	195 K.	7 mo.	—	T_1	June 20	8·68	20·3	9,500	85	—	12	3	1	—
	196 K.	7 mo.	11.55 a.m.	T_1	June 20	10·19	19·4	13,900	80	5	13	2	1	—
	†196 K.‡	—	12.05 p.m.	A_1	June 20	—	—	4,500	41	1	5	3	1	—
	197 K.	7 mo.	—	T_1	June 20	8·40	19·64	18,000	70	4	21	5	—	—
	197 K.	—	—	T_2	June 20	—	18·54	13,900	86	2	5	7	3	—
	198 K.	7 mo.	—	T_1	June 20	10·20	17·2	12,900	—	—	—	—	—	—
Complete, Greenhouse.....	61 K.	5 mo.	10.05 a.m.	T_1	Apr. 24	10·30	19·6	25,400	57	3	37	3	—	—
	61 K.	—	10.07 a.m.	T_2	Apr. 24	—	17·8	24,900	—	—	—	—	—	—
	74 K.	5 mo.	11.45 a.m.	T_1	Apr. 29	10·33	17·5	14,400	81	3	13	3	—	—
	73 K.	5 mo. +	11.05 a.m.	T_1	Apr. 29	10·57	17·0	10,500	89	3	6	2	—	—
	§71 K.	5 mo.	10.10 a.m.	A_1	Apr. 29	8·81	—	6,300	69	10	17	4	—	—

Abbreviations.—T_1 = First sample of blood from tail. T_2 = Second sample of blood from tail. Mo. = Months. A = Aorta.

* Killed May 29th. † Only 50 cells counted. ‡ Killed June 20th. § Killed April 29th.

SWISS STRAIN-MICE—1 YEAR.

Diet.	Experimental No.	Age.	Time.	Aorta Tail.	Date Bled.	Red Blood Count.	Haemoglobin.	White Blood Count.	Lymphocytes.	Monocytes.	Neutrophiles.	Eosinophiles.	Degenerated Cells.	Doubtful.
					1930.		Gm. per 100 cc.							
Standard	32 K.	13 mo.	10.25 a.m.	T_1	Apr. 10	11·34	21·52	16,800	63	4	30	3	1	—
	* 52 K.	12 mo.	11.05 a.m.	A_1	Apr. 21	9·92	—	10,500	—	—	—	—	—	—
	100 K.	14 mo.	9.48 a.m.	T_1	May 13	10·18	17·5	5,300	82	—	16	2	3	—
	100 K.	—	9.51 a.m.	T_2	May 13	—	17·5	5,400	—	—	—	—	—	—
	134 K.	14 mo.	11.35 a.m.	T_1	May 26	11·0	—	—	61	4	32	3	—	—
	†134 K.	—	—	A_1	May 26	9·97	—	—	67	3	24	6	3	—
Complete	34 K.	13 mo.	11.35 a.m.	T_1	Apr. 10	9·5	18·55	10,000	53	5	31	11	5	—
	‡ 72 K.	13 mo.	10.20 a.m.	A_1	Apr. 29	9·59	18·2	1,500	9	5	8	3	—	—
	102 K.	14 mo.	11.10 a.m.	T_1	May 13	9·72	18·5	11,100	56	8	35	1	2	—
	102 K.	—	11.13 a.m.	T_2	May 13	—	17·5	9,600	50	6	43	1	—	—
Sherman	31 K.	13 mo.	10.15 a.m.	T_1	Apr. 10	11·1	22·4	22,100	80	2	18	—	3	—
	97 K.	14 mo.	2.20 p.m.	T_1	May 12	10·17	19·3	11,400	72	6	17	5	—	—
Sherman + Garlic	38 K.	12 mo.	11.05 a.m.	T_1	Apr. 12	10·75	17·56	7,200	59	2	38	1	—	—
	87 K.	14 mo.	1.35 p.m.	T_1	May 6	9·60	17·8	12,700	63	6	29	2	3	—
	87 K.	—	1.38 p.m.	T_2	May 6	—	—	8,500	—	—	—	—	—	—
Sherman + Sugar	41 K.	13 mo.	10.46 a.m.	T_1	Apr. 14	10·46	14·78	25,200	67	4	25	4	10	—
	93 K.	13 mo.	1.45 p.m.	T_1	May 8	9·80	20·5	23,100	70	3	26	1	—	—
	93 K.	—	1.48 p.m.	T_2	May 8	—	19·1	19,800	—	—	—	—	—	—
	122 K.	14 mo.	10.00 a.m.	T_1	May 21	9·74	—	13,000	65	8	24	3	—	—
	§ —	—	—	A_1	May 21	9·77	—	3.600	70	6	21	3	9	—
Sherman + Tar Oil	43 K.	12 mo.	11.15 a.m.	T_1	Apr. 15	10·47	20·76	13,900	76	5	16	3	6	—
	43 K.	—	11.20 a.m.	T_2	Apr. 15	10·56	19·85	9,350	—	—	—	—	—	—
	89 K.	13 mo.	11.15 a.m.	T_1	May 7	8·29	16·15	10,900	59	4	31	6	4	—
	89 K.	—	11.18 a.m.	T_2	May 7	—	15·2	10,300	—	—	—	—	—	—
	121 K.	14 mo.	9.40 a.m.	T_1	May 21	8·27	17·4	9,430	85	3	10	2	—	—
	§121 K.	—	—	A_1	May 21	9·88	—	3,500	20	2	2	1	2	—

Abbreviations.—T_1 = First sample of blood from tail. T_2 = Second sample of blood from tail. Mo. = Months. A = Aorta.
* Killed April 21st. † Killed May 26th. ‡ Killed April 29th. § Killed May 21st.

SWISS STRAIN-MICE—1 YEAR—(*continued*).

Diet.	Experimental No.	Age.	Time.	Aorta Tail.	Date Bled.	Red Blood Count.	Haemoglobin	White Blood Count.	Lymphocytes.	Monocytes.	Neutrophiles.	Eosinophiles.	Degenerated Cells.	Doubtful.
					1930.		Gm. per 100 cc.							
Sherman Meat and Potatoes	42 K.	11 mo.	10.00 a.m.	T_1	Apr. 15	9·37	19·85	17,100	55	10	28	7	3	—
	42 K.	—	10.05 a.m.	T_2	Apr. 15	9·56	20·00	6,300	—	—	—	—	—	—
	76 K.	—	—	—	—	—	—	—	—	—	—	—	—	—
	91 K.	11 mo.	1.58 p.m.	T_1	May 8	8·52	18·0	10,500	52	1	47	—	—	—
	94 K.	—	2.01 p.m.	T_2	May 8	—	17·2	10,500	—	—	—	—	—	—
	126 K.	12 mo.	9.30 a.m.	T_1	May 22	9·90	—	10,500	77	12	10	1	4	—
	*126 K.	—	—	A_1	May 22	8·03	—	6,600	68	6	24	2	6	—
	129 K.	12 mo.	9.25 a.m.	T_1	May 23	9·05	—	28,400	69	13	16	2	—	—
Sherman Fat	129 K.	—	9.35 a.m.	A_1	May 23	8·27	—	9,200	66	16	16	2	3	—
	†146 K.	12 mo.	2.15 p.m.	T_1	May 28	11·33	—	4,300	47	1	7	1	6	—
	146 K.	—	2.18 p.m.	T_2	May 28	—	—	4,100	—	—	—	—	—	—
	147 K.	12 mo.	2.20 p.m.	T_1	May 29	9·21	19·0	13,300	80	1	18	1	4	—
	147 K.	—	2.23 p.m.	T_2	May 29	—	19·4	10,600	—	—	—	—	—	—
Sherman Arsenic	40 K.	13 mo.	2.00 p.m.	T_1	Apr. 14	10·06	15·64	11,950	38	6	38	18	4	—
	98 K.	13 mo.	2.40 p.m.	T_1	May 12	10·20	17·5	17,900	78	4	14	4	—	—
	—	—	—	T_2	May 12	—	—	14,100	—	—	—	—	—	—
Sherman Salts, Experiment No. 2	44 K.	12 mo.	2.00 p.m.	T_1	Apr. 15	10·52	19·00	13,900	73	2	24	1	1	—
	96 K.	13 mo.	11.30 a.m.	T_1	May 12	9·11	15·7	22,200	82	4	14	—	—	—
	—	—	11.33 a.m.	T_2	May 12	—	—	21,400	—	—	—	—	—	—
Sherman Coal Tar (Aqueous)	45 K.	13 mo.	2.30 p.m.	T_1	Apr. 15	10·00	20·57	13,350	72	—	25	3	1	—
	45 K.	—	2.35 p.m.	T_2	May 15	—	18·54	12,100	—	—	—	—	—	—
	90 K.	13 mo.	11.30 a.m.	T_1	May 7	9·25	17·2	8,900	80	4	6	10	6	—
	90 K.	—	11.33 a.m.	T_2	May 7	—	16·3	8,100	—	—	—	—	—	—
Sherman Alcohol	48 K.	11 mo.	10.00 a.m.	T_1	Apr. 17	11·92	21·92	24,300	74	7	18	1	—	—
	48 K.	—	10.03 a.m.	T_2	Apr. 17	—	—	16,700	—	—	—	—	—	—
	‡48 K.	—	—	A_1	Apr. 17	—	16·05	4,200	80	12	7	1	—	—
	95 K.	11 mo.	11.20 a.m.	T_1	May 12	10·79	19·6	20,400	86	1	10	3	—	—
	95 K.	—	—	T_2	May 12	—	—	21,600	—	—	—	—	—	—
	149 K.	12 mo.	11.45 a.m.	T_1	June 2	10·22	21·4	13,500	76	7	13	4	5	—
	149 K.	—	—	T_2	June 2	—	21·1	10,600	—	—	—	—	—	—

Abbreviations: T_1 = First sample of blood from tail. T_2 = Second sample of blood from tail. Mo. = Months. A = Aorta.

* Killed May 22nd. † Only 50 cells counted. ‡ Killed April 17th.

SWISS STRAIN-MICE—2 YEARS.

Diet.	Experimental No.	Age.	Time.	Aorta Tail.	Date Bled.	Red Blood Count.	Haemoglobin.	White Blood Count.	Lymphocytes.	Monocytes.	Neutrophiles.	Eosinophiles.	Degenerated Cells.	Doubtful.
					1930.		Gm. per 100 cc.							
Standard	46 K.	24 mo.	11.25 a.m.	T_1	Apr. 16	9·80	17·0	20,500	73	8	19	—	1	—
	46 K.	—	11.30 a.m.	T_2	Apr. 16	—	20·7	18,700	—	—	—	—	—	—
	103 K.	24 mo.	2.10 p.m.	T_1	May 13	10·28	—	13,200	48	5	35	12	—	—
	103 K.	—	2.13 p.m.	T_2	May 13	—	—	12,600	—	—	—	—	—	—
	125 K.	24 mo.	9.10 a.m.	T_1	May 22	9·10	—	10,400	75	2	19	4	3	—
	*125 K.	—	—	A_1	May 22	—	—	—	78	8	11	3	8	—
Complete	47 K.	24 mo.	2.10 p.m.	T_1	Apr. 16	10·86	18·4	12,600	40	4	51	5	3	—
	††47 K.	—	2.15 p.m.	T_2	Apr. 16	—	18·5	11,500	—	—	—	—	—	—
	99 K.	24 mo.	9.35 a.m.	T_1	May 13	9·25	18·2	19,800	33	—	66	1	—	—
	99 K.	—	9.38 a.m.	T_2	May 13	—	17·9	10,900	—	—	—	—	—	—
Sherman	51 K.	24 mo.	10.50 a.m.	T_1	Apr. 19	9·05	16·8	10,300	88	8	2	—	1	1 2
	51 K.	—	10.25 a.m.	T_2	Apr. 19	—	16·05	9,900	—	—	—	—	—	—
	104 K.	24 mo.	2.25 p.m.	T_1	May 13	9·11	—	9,000	—	—	—	—	—	—
	104 K.	—	2.28 p.m.	T_2	May 13	—	—	9,100	—	—	—	—	—	—
Sherman + Meat and Potatoes	49 K.	24 mo.	2.15 p.m.	T_1	Apr. 17	12·62	24·7	13,100	82	2	11	5	—	—
	108 K.	24 mo.	10.45 a.m.	T_1	May 15	9·69	15·3	39,400	72	6	14	8	—	—
	108 K.	—	—	T_2	May 15	—	—	33,800	—	—	—	—	—	—
	109 K.	24 mo.	2.23 p.m.	T_1	May 15	9·19	15·3	18,300	60	5	34	1	1	—
	109 K.	—	2.26 p.m.	T_2	May 15	—	13·7	14,100	—	—	—	—	—	—
Sherman + Arsenic	50 K.	24 mo.	2.55 p.m.	T_1	Apr. 17	8·47	16·2	10,300	63	4	26	7	3	—
	50 K.	—	2.58 p.m.	T_2	Apr. 17	—	15·4	8,650	—	—	—	—	—	—
	101 K.	24 mo.	10.50 a.m.	T_1	May 13	11·07	20·7	7,000	78	2	20	—	3	—
	101 K.	—	—	T_2	May 13	—	19·4	6,200	—	—	—	—	—	—

Abbreviations.—T_1 = First sample of blood from tail. T_2 = Second sample of blood from tail. Mo. = Months. A = Aorta.
* Killed May 22nd. † Autopsy 2551. ‡ Died April 17th.

ROCKEFELLER MICE: 3 MONTHS.

Time of Bleeding.	Experimental No.	Red Blood Count.	Haemoglobin.	White Blood Count.	Lymphocytes.	Monocytes.	Neutrophiles.	Eosinophiles.	Degenerated Cells.	Died.
			Gm. per 100 cc.							
11.30 a.m.	157 K.	9·04	19·9	16,800	58	6	24	12	1	Complete.
11.33 a.m.	157 K.	—	19·6	16,200	—	—	—	—	—	Complete.
11.40 a.m.	158 K.	9·56	19·6	15,800	61	9	21	9	—	Complete.
—	158 K.	—	19·4	14,100	—	—	—	—	—	Complete.
2.40 p.m.	159 K.	10·46	22·3	9,500	74	7	14	5	1	Complete.
2.43 p.m.	159 K.	—	22·3	7,700	—	—	—	—	—	Complete.
2.55 p.m.	160 K.	10·41	20·9	11,400	80	2	15	3	3	Complete.
—	160 K.	—	20·3	9,700	—	—	—	—	—	Complete.

ROCKEFELLER MICE: 7 MONTHS.

Diet.	Experimental No.	Age.	Time.	Aorta Tail.	Date Bled.	Red Blood Count.	Haemoglobin.	White Blood Count.	Lymphocytes.	Monocytes.	Neutrophiles.	Eosinophiles.	Degenerated Cells
					1930.		Gm. per 100 cc.						
Standard	115 K.	7 mo.	1.50 p.m.	T_1	May 19	9·72	17·6	7,500	60	—	36	4	1
	115 K.	—	1.53 p.m.	T_2	May 19	—	15·7	6,000	—	—	—	—	—
	132 K.	7 mo.	2.30 p.m.	T_1	May 23	11·37	17·4	24,600	63	9	24	4	—
	132 K.	—	—	T_2	May 23	—	17·0	14,800	—	—	—	—	—
	199 K.	8 mo.	2.00 p.m.	T_1	June 23	9·97	21·9	11,300	77	4	19	—	1
	*199 K.	8 mo.	2.20 p.m.	A_1	June 23	10·57	—	2,530	39	4	4	3	3
	200 K.	8 mo.	2.10 p.m.	T_1	June 23	10·06	21·7	16,100	60	5	18	3	3
	*200 K.	8 mo.	2.35 p.m.	A_1	June 23	9·95	—	3,700	43	2	2	3	3
Complete	114 K.	7 mo.	1.35 p.m.	T_1	May 19	11·35	20·7	16,400	85	2	12	1	—
	114 K.	—	1.38 p.m.	T_2	May 19	—	22·7	13,800	—	—	—	—	—
	131 K.	7 mo.	2.15 p.m.	T_1	May 23	9·43	17·9	15,020	72	8	16	4	1
	131 K.	—	2.18 p.m.	T_2	May 23	—	17·4	11,300	—	—	—	—	—
Sherman + Tar Oil	155 K.	8 mo.	12.05 p.m.	T_1	June 3	10·8	21·7	16,900	79	7	14	—	8
	155 K.	—	12.08 p.m.	T_2	June 3	—	—	14,100	—	—	—	—	—
	156 K.	8 mo.	2.40 p.m.	T_1	June 3	10·56	19·1	16,600	58	8	30	4	26
	156 K.	—	—	T_2	—	—	20·7	8,500	51	5	35	9	17
Sherman + Meat and Potatoes	120 K.	6 mo. +	2.25 p.m.	T_1	May 20	10·81	19·47	21,900	61	0	28	11	8
	120 K.	—	—	T_2	May 20	—	21·49	14,700	—	—	—	—	—
Sherman + Salts, Experiment No. 2	116 K.	6 mo.	3.05 p.m.	T_1	May 19	9·74	18·6	11,700	79	2	14	5	11
	116 K.	—	3.08 p.m.	T_2	—	—	18·6	10,400	—	—	—	—	—
	139 K.	7 mo.	10.30 a.m.	T_1	May 27	10·11	20·5	11,500	71	5	14	11	4
	139 K.	—	10.33 a.m.	T_2	May 27	—	19·8	8,900	—	—	—	—	—
Sherman + Raw Meat	119 K.	7 mo.	2.05 p.m.	T_1	May 20	10·8	20·54	12,600	62	2	30	6	—
	119 K.	—	—	T_2	May 20	—	21·3	9,100	—	—	—	—	—
	142 K.	6 mo. +	2.15 p.m.	T_1	May 27	11·25	19·1	9,200	35	3	6	6	2
	142 K.	—	2.18 p.m.	T_2	May 27	—	18·0	6,200	—	—	—	—	—
Sherman + Alcohol	113 K.	6 mo. +	11.30 a.m.	T_1	May 17	10·91	22·1	18,200	80	1	16	3	5
	113 K.	—	11.33 a.m.	T_2	May 17	—	—	13,200	—	—	—	—	—
	140 K.	7 mo. +	10.55 a.m.	T_1	May 27	10·23	17·9	15,600	66	6	19	9	1
	140 K.	—	—	T_2	May 27	—	17·5	10,100	—	—	—	—	—
	152 K.	6 mo. +	12.00 noon	T_1	June 2	9·87	20·7	16,900	65	3	21	11	3
	152 K.	—	12.03 p.m.	T_2	June 2	—	—	11,600	—	—	—	—	—
	165 K.	6 mo. +	11.10 a.m.	T_1	June 6	10·31	20·3	13,600	78	4	13	5	—
	165 K.	—	—	T_2	June 6	—	22·1	10,100	—	—	—	—	—
	166 K.	6 mo. +	11.20 a.m.	T_1	June 6	9·2	21·7	14,000	73	4	19	4	1
	166 K.	—	—	T_2	June 6	—	18·5	10,100	—	—	—	—	—
	167 K.	6 mo. +	1.45 p.m.	T_1	June 6	10·02	19·0	14,000	70	4	24	2	12
	167 K.	—	—	T_2	June 6	—	19·4	11,600	—	—	—	—	—
	168 K.	6 mo. +	1.55 p.m.	T_1	June 6	9·36	18·5	12,900	71	5	16	8	11
	168 K.	—	—	T_2	June 6	—	17·4	11,600	—	—	—	—	—

Abbreviations.—T_1 = First sample of blood from tail. T_2 = Second sample of blood from tail. Mo. = Months. A = Aorta.
* Killed June 23rd.

Diet.	Experimental No.	Age.	Time.	Aorta Tail.	Date Bled.	Red Blood Count.	Haemoglobin.	White Blood Count.	Lymphocytes.	Monocytes.	Neutrophiles.	Eosinophiles.	Degenerated Cells.	Doubtful.
					1930.		Gm. per 100 cc.							
Standard	36 K.	14 mo.	2.00 p.m.	T_1	Apr. 11	10·07	17·4	13,200	59	9	26	6	8	—
	105 K.	15 mo.	2.20 p.m.	T_1	May 14	10·12	17·5	10,600	67	1	29	3	1	—
	—	—	—	T_2	May 14	—	—	8,500	—	—	—	—	—	—
Complete	57 K.	12 mo.	12.05 p.m.	T_1	Apr. 22	9·55	15·2	10,000	24	22	41	13	—	—
	57 K.	—	12.09 p.m.	T_2	Apr. 22	—	15·7	8,300	23	29	33	15	—	—
	106 K.	14 mo.	2.35 p.m.	T_1	May 14	10·03	15·9	17,500	42	6	50	2	—	—
	106 K.	—	—	T_2	May 14	—	—	15,100	—	—	—	—	—	—
	161 K.	12 mo. +	9.35 a.m.	T_1	June 5	9·72	20·3	13,100	40	10	42	6	6	2
	161 K.	—	—	T_2	—	—	19·3	10,100	—	—	—	—	—	—
	162 K.	12 mo.	9.45 a.m.	T_1	June 5	10·32	19·6	13,300	74	2	20	4	15	—
	162 K.	—	—	T_2	June 5	—	18·8	7,400	—	—	—	—	—	—
	163 K.	12 mo. +	10.55 a.m.	T_1	June 5	9·52	17·5	9,400	55	7	33	4	13	1
	163 K.	—	—	T_2	June 5	—	16·7	9,000	—	—	—	—	—	—
	164 K.	12 mo. +	11.05 a.m.	T_1	June 5	9·7	19·1	13,700	91	3	6	—	—	—
	164 K.	—	—	T_2	June 5	—	18·3	10,400	—	—	—	—	—	—
Sherman + Tar Oil	35 K.	15 mo.	10.30 a.m.	T_1	Apr. 11	9·81	19·6	14,000	70	11	17	2	—	—
	35 K.	—	—	A_1	Apr. 11	7·42	—	4,000	—	—	—	—	—	—
	55 K.	13 mo.	10.22 a.m.	T_1	Apr. 22	9·61	14·9	14,100	67	9	23	1	4	—
	55 K.	—	10.25 a.m.	T_2	Apr. 22	—	15·2	14,100	—	—	—	—	—	—
Sherman + Meat and Potatoes	59 K.	13 mo.	1.45 p.m.	T_1	Apr. 23	10·48	17·9	8,560	66	1	25	8	—	—
	59 K.	—	1.48 p.m.	T_2	Apr. 23	—	16·6	7,400	—	—	—	—	—	—
	107 K.	14 mo.	10.30 a.m.	T_1	May 15	9·49	15·9	8,800	76	14	8	2	1	—
	107 K.	—	10.33 a.m.	T_2	May 15	—	15·5	6,900	—	—	—	—	—	—
	130 K.	14 mo. +	9.30 a.m.	T_1	May 23	9·64	—	6,700	75	11	6	8	3	—
	*130 K.	—	9.40 a.m.	A_1	May 23	9·42	—	3,500	72	13	11	4	few	—
Sherman + Salts, Experiment No. 2	56 K.	13 mo.	11.20 a.m.	T_1	Apr. 22	9·92	18·4	10,000	56	4	36	4	1	—
	56 K.	—	11.23 a.m.	T_2	Apr. 22	—	15·8	9,900	—	—	—	—	—	—
	58 K.	14 mo.	2.30 p.m.	T_1	Apr. 22	9·16	15·7	15,500	76	1	14	9	—	—
	—	—	2.33 p.m.	T_2	Apr. 22	—	15·7	15,300	—	—	—	—	—	—
Sherman + Alcohol	37 K.	14 mo.	2.10 p.m.	T_1	Apr. 11	8·33	14·2	17,600	42	28	29	1	—	—
	39 K.	14 mo.	11.00 a.m.	A_1	Apr. 14	6·87	11·68	12,000	55	24	19	2	3	—
	60 K.	12 mo.	2.30 p.m.	T_1	Apr. 23	12·23	19·0	12,400	64	8	16	12	—	—
	60 K.	—	2.33 p.m.	T_2	Apr. 23	—	16·2	11,600	—	—	—	—	—	—
	154 K.	13 mo.	11.55 a.m.	T_1	June 3	10·27	19·1	15,400	74	10	10	6	15	—
	154 K.	—	11.58 a.m.	T_2	June 3	—	20·7	12,100	—	—	—	—	—	—

Abbreviations.—T_1 = First sample of blood from tail. T_2 = Second sample of blood from tail. Mo. = Months. A = Aorta.
* Killed May 23rd.

APPENDIX II.—*Blood Counts for two strains of Mice at various ages and different diets arranged according to time of bleeding.*

SWISS MICE: 2-3-4 MONTHS.

Time of Bleeding.	Experimental No.	Red Blood Counts.	Haemoglobin.	White Blood Counts.	Lymphocytes.	Monocytes.	Neutrophiles.	Eosinophiles.	Degenerated Cells.	Diet.
			Gm. per 100 cc.							
9.40 a.m.	22 K.	9·62	21·9	13,400	60	10	30	—	3	Greenhouse, Complete.
10.40 a.m.	17 K.	8·47	20·0	9,400	61	3	32	4	—	Sherman + Garlic.
10.55 a.m.	10 K.	10·07	21·3	17,400	80	5	14	1	—	+ Raw Meat.
11.00 a.m.	62 K.	10·57	18·7	11,900	79	2	19	—	1	Complete.
11.04 a.m.	62 K.	—	—	11,500	—	—	—	—	—	—
11.30 a.m.	14 K.	9·66	20·35	25,000	90	1	5	—	4	Sherman.
11.35 a.m.	65 K.	10·01	17·66	10,000	84	1	12	3	6	Sherman.
11.38 a.m.	65 K.	—	—	10,300	—	—	—	—	—	+ Garlic.
11.40 a.m.	19 K.	9·95	21·5	13,900	80	3	13	4	—	+ Sugar.
11.30 a.m.	133 K.	10·46	—	11,700	—	—	—	—	—	+ Sugar.
—	133 K.	8·55	—	6,830	—	—	—	—	—	—
11.10 a.m.	18 K.	9·44	19·6	19,200	77	1	18	4	—	+ Tar Oil.
11.05 a.m.	13 K.	10·00	22·2	23,300	96	—	3	1	—	+ Fat.
11.00 a.m.	68 K.	9·69	20·3	24,100	86	1	10	3	—	+ Fat.
11.03 a.m.	68 K.	—	—	21,900	—	—	—	—	—	—
11.40 a.m.	33 K.	7·95	17·5	14,300	80	8	11	1	4	+ Arsenic.
2.40 p.m.	12 K.	10·08	20·0	25,400	77	5	17	—	—	Standard.
2.40 p.m.	21 K.	9·14	21·1	13,100	79	1	22	3	6	Complete.
2.35 p.m.	64 K.	8·99	15·8	8,200	—	—	—	—	—	Sherman.
2.39 p.m.	64 K.	—	15·2	7,070	—	—	—	—	—	—
2.55 p.m.	67 K.	10·43	19·0	19,500	88	2	6	4	—	+ Sugar.
2.59 p.m.	67 K.	—	16·9	—	—	—	—	—	—	—
1.45 p.m.	66 K.	10·65	19·3	10,400	73	4	21	2	—	+ Tar Oil.
1.48 p.m.	66 K.	—	19·0	8,900	—	—	—	—	—	—
2.15 p.m.	20 K.	10·19	17·2	6,700	39	—	2	—	—	+ Meat and Potatoes.
2.48 p.m.	76 K.	8·82	16·5	7,900	70	3	24	3	—	+ Meat and Potatoes.
1.25 p.m.	77 K.	9·67	19·8	9,700	79	1	21	4	—	+ Arsenic.
1.28 p.m.	77 K.	—	—	6,500	—	—	—	—	—	—
2.20 p.m.	15 K.	9·81	20·0	19,300	86	—	14	—	—	+ Salts, Experiment No. 2.
1.50 p.m.	69 K.	10·58	20·3	16,600	82	7	10	1	—	+ Salts, Experiment No. 2.
2.40 p.m.	16 K.	15·08	21·3	33,900	95	—	3	2	—	+ Aqueous Tar.
2.45 p.m.	92 K.	10·6	16·8	12,300	85	3	9	3	—	+ Aqueous Tar.
2.15 p.m.	81 K.	10·01	21·5	17,800	88	4	5	3	5	+ Alcohol.
2.20 p.m.	81 K.	—	—	16,800	—	—	—	—	—	+ Alcohol.
3.10 p.m.	54 K.	10·08	20·1	9,800	92	—	4	4	—	+ Alcohol.
3.13 p.m.	54 K.	—	—	13,200	—	—	—	—	—	—

SWISS MICE: 5–6–7 MONTHS.

Time of Bleeding.	Experimental No.	Red Blood Counts.	Haemoglobin.	White Blood Counts.	Lymphocytes.	Monocytes.	Neutrophiles.	Eosinophiles.	Degenerated Cells.	Doubtful.	Diet.
			Gm. per 100 cc.								
9.25 a.m.	138 K.			—	17	6	11	6	9		Standard.
9.40 a.m.	138 K.			1,250	45	2 1 2	21 3	1	2 1 2		Standard.
9.15 a.m.	137 K.			2,800	91			4			Sherman.
9.30 a.m.	A137 K.	8·73		9,100	47	13	13	1			Sherman.
9.50 a.m.	143 K.	7·96		3,200	73	5 5	11	5 2			Sugar.
9.25 a.m.	A143 K.	10·16		12,900	29		21				—
	177 K.		18·2	12,400	70				2		Sherman.
9.40 a.m.	177 K.	8·44	18·3	11,300		4	24	3	3		Sherman.
	178 K.		18·9	17,700	69						Sherman.
9.40 a.m.	178 K.	9·69	18·3	15,200		4	11	5	2 3		Fat.
9.25 a.m.	185 K.	9·23	20·3	14,900	80	4	7	2			Arsenic.
9.45 a.m.	207 K.		19·3	11,200	87				2 3		Arsenic.
9.45 a.m.	208 K.	9·77	20·7	13,500	81	9	6	4	1		Arsenic.
9.48 a.m.	208 K.		20·3	11,600							Arsenic.
9.55 a.m.	201 K.	8·35	21·7	9,800	79	5	13	3	9		Tar Oil.
9.50 a.m.	2 K.	9·96	20·7	12,300	70		25	4	1		Standard.
10.15 a.m.	23 K.	9·96	19·3	21,700	93		7				Complete.
10.10 a.m.	1 K.	10·36	22·8	18,300	42	2 1 3	53	1	1	2	Sherman.
10.40 a.m.	9 K.	9·48	20·36	17,400	76		18	2 1 3			Arsenic.
10.05 a.m.	61 K.	10·30	19·6	25,400	57		37	3			Complete Greenhouse.
10.07 a.m.	61 K.		17·8	24,900							Complete Greenhouse.
10.10 a.m.	71 K.	8·81		6,300	69	10	17	4	15		Standard.
10.20 a.m.	169 K.			20,300	84	4	9	2 1			Standard.
10.30 a.m.	170 K.	9·75	18·7	22,000	83	9	3	5	3		Standard.
10.50 a.m.	171 K.	11·27	17·8	15,400	80	4	14	6			Sherman.
10.50 a.m.	179 K.	10·18		14,700	79	2 3	15	3			Fat.
10.10 a.m.	186 K.	9·64	19·4	19,600	76	3	5	4			Fat.
*10.20 a.m.	A186 K.	9·60		7,900	37	1	5	1	5		Salts, Experiment No. 2.
10.05 a.m.	192 K.	8·55	20·7	13,300	86	5	10	2 1			Salts, Experiment No. 2.
10.10 a.m.	A192 K.			3,800	83	1					Salts, Experiment No. 2.
10.20 a.m.	A193 K.	8·67	18·8	6,700	67	2	23	3			Salts, Experiment No. 2.
10.30 a.m.	193 K.	8·05		2,100	79		13	1	2 1 2		Salts, Experiment No. 2.
*10.25 a.m.	193 K.	9·99	19·6	3,800	40	1	9	1	1 3		Salts, Experiment No. 2.
10.05 a.m.	202 K.	8·61	22·3	9,800	52	6	36	5	3 2		Tar Oil.
10.15 a.m.	A202 K.	8·31		4,970	65	6	27	2	2 1		Tar Oil.
10.25 a.m.	209 K.	8·91	22·5	14,700	64	4	24	4	2 1		Arsenic.
10.28 a.m.	209 K.		21·1	12,900							Arsenic.
10.35 a.m.	210 K.	10·72	20·5	13,300	81	4	11	4			Arsenic.
10.38 a.m.	210 K.		17·9	14,100							Tar Oil.
11.15 a.m.	203 K.	8·67	19·3	12,100	69	8 3	14	9			Tar Oil.
11.25 a.m.	A203 K.	8·30		21,200	71		20	6			Tar Oil.

* Only 50 cells counted.

SWISS MICE: 5-6-7 MONTHS—(*continued*).

Time of Bleeding.	Experimental No.	Red Blood	Haemoglobin.	White Blood Counts.	Lymphocytes.	Monocytes.	Neutrophiles.	Eosinophiles.	Degenerated Cells.	Doubtful.	Diet.
			Gm. per 100 cc.								
11.45 a.m.	63 K.	10·46	15·3	16,600	81	4	11	4	—	—	Standard.
11.48 a.m.	63 K.	—	17·5	19,800	—	—	—	—	—	—	Standard.
11.45 a.m.	25 K.	9·36	20·3	8,300	87	—	11	—	2	—	Standard.
11.15 a.m.	24 K.	9·08	19·4	6,350	94	—	4	—	—	—	Sherman.
11.05 a.m.	5 K.	9·23	14·7	11,400	75	12	12	1	—	—	Sugar.
11.20 a.m.	6 K.	9·58	20·1	15,600	72	6	18	4	—	—	Tar Oil.
11.30 a.m.	28 K.	—	—	—	—	—	—	—	—	—	—
11.50 a.m.	84 K.	10·37	19·6	12,500	71	1	18	8	7	—	Meat and Potatoes.
11.15 a.m.	27 K.	10·92	23·6	10,900	90	3	3	4	—	—	Fat.
11.30 a.m.	83 K.	11·18	19·9	12,600	55	0	34	11	—	—	Arsenic.
11.33 a.m.	83 K.	—	19·9	9,500	—	—	—	—	—	—	Arsenic.
11.00 a.m.	150 K.	10·07	—	16,400	90	—	8	2	3	—	Aqueous Tar.
*11.10 a.m.	A150 K.	9·47	—	1,400	34	11	2	3	1	—	Aqueous Tar.
11.45 a.m.	74 K.	10·33	17·5	14,400	81	3	13	3	—	—	Complete Greenhouse.
11.05 a.m.	73 K.	10·57	17·0	10,500	89	3	6	2	—	—	Complete Greenhouse.
11.15 a.m.	173 K.	12·22	24·7	14,000	72	4	23	1	—	—	Complete.
—	173 K.	—	24·5	8,800	—	—	—	—	—	—	Complete.
11.25 a.m.	174 K.	10·45	22·9	12,300	75	2	22	1	6	—	Complete.
—	174 K.	—	22·8	11,000	—	—	—	—	—	—	Complete.
11.10 a.m.	180 K.	9·06	19·0	10,800	78	5	12	5	1	—	Sherman.
—	180 K.	—	18·5	7,800	—	—	—	—	—	—	Sherman.
11.20 a.m.	181 K.	9·91	20·3	15,200	84	4	11	1	2	—	Garlic.
—	181 K.	—	19·9	13,600	—	—	—	—	—	—	Garlic.
11.35 a.m.	182 K.	9·77	20·5	4,200	26	7	16	1	—	—	Garlic.
—	182 K.	—	20·0	5,500	—	—	—	—	—	—	Garlic.
11.25 a.m.	187 K.	10·11	20·9	15,800	80	5	11	4	24	—	Fat.
11.30 a.m.	A187 K.	9·13	—	4,100	42	4	3	1	8	—	—
11.10 a.m.	188 K.	10·18	22·5	12,700	76	3	17	4	—	—	Sugar.
11.23 a.m.	189 K.	9·01	23·4	19,500	49	5	31	15	—	—	Sugar.
11.26 a.m.	189 K.	—	20·3	13,300	—	—	—	—	—	—	Sugar.
11.45 a.m.	194 K.	8·70	19·8	12,700	78	6	12	4	—	—	Salts, Experiment No. 2.
11.55 a.m.	A194 K.	8·00	—	3,000	87	5	7	1	—	—	—
11.40 a.m.	195 K.	8·68	—	9,500	85	12	3	1	—	—	Alcohol.
11.55 a.m.	196 K.	10·19	—	13,900	80	5	13	2	1	—	Alcohol.
*12.05 p.m.	A196 K.	—	—	4,500	41	1	5	3	1	—	Alcohol.
11.30 a.m.	204 K.	9·64	21·7	12,700	83	2	14	1	2	—	Meat and Potatoes.
11.33 a.m.	204 K.	—	—	11,500	—	—	—	—	—	—	Meat and Potatoes.
11.15 a.m.	213 K.	10·69	22·3	20,000	78	5	14	2	—	—	Raw Meat.
11.18 a.m.	213 K.	—	22·3	20,200	—	—	—	—	—	—	Raw Meat.
11.25 a.m.	214 K.	9·72	21·1	18,000	81	4	12	3	—	—	Raw Meat.
11.28 a.m.	214 K.	—	21·3	15,800	—	—	—	—	—	—	Raw Meat.

* Only 50 cells counted.

SWISS MICE: 5-6-7 MONTHS—(*continued*).

Time of Bleeding.	Experimental No.	Red Blood Corpuscles.	Haemoglobin.	White Blood Corpuscles.	Lymphocytes.	Monocytes.	Neutrophiles.	Eosinophiles.	Degenerated Cells.	Doubtful.	Diet.
			Gm. per 100 cc.								
1.50 p.m.	111	10	20·3	15,100	13	4	15	6			Standard.
1.55 p.m.	111	9	17·9	13,000	20	1	25	1	2		Standard.
1.30 p.m.	114	9	21	13,500	31	4	36	7			Salts. Experiment No. 2
1.55 p.m.	114	7	17	11,600	51	4	37	7			Salts. Experiment No. 2
1.55 p.m.	110	9	16·6	14,000	33	6	45	3			Raw Meat.
1.50 p.m.	112	12	16·6	12,400	39	4	45	3	1		Raw Meat.
1.50 p.m.	113	10	19·4	20,200	35	2	36	3			Standard.
1.45 p.m.	115	9	21·1	16,100	34	4	37	3			Standard.
1.30 p.m.	116	9	21·3	14,800	34	1	20	3	1		Garlic.
1.55 p.m.	117	10	20·9	17,300	26	1	20	3			Raw Meat.
1.55 p.m.	118	10	21·6	14,300	27	2	14	6			Standard.
2.0 p.m.	119	10	21·6	21,600	27	3	14	9	3		Complete.
2.0 p.m.	120	10	19·6	15,700	27	3	23	3	3		Complete.
2.0 p.m.	121	9	21	16,200	28	3	16	3	4		Sherman.
2.0 p.m.	122	10	21	15,200	27	3	13	6			Sherman.
2.0 p.m.	123	10	24		24	3	21	2			Garlic.
2.0 p.m.	124	9	16	6,300	24	3	13	6	1		Garlic.
2.0 p.m.	125	9	15·3	9,200	24	3	23	3			Garlic.
2.0 p.m.	126	9	16·3	10,500	24	3	20	3			Garlic.
2.0 p.m.	127	9	15·3	9,500	24	3	20	3			Tar Oil.
2.0 p.m.	128	9	16	12,500	24	3	20	3	1		Meat and Potatoes.
2.0 p.m.	129	10	16	13,000	24	3	22	1			Fat.
2.0 p.m.	130	10	17	12,000	24	3	22	1			Fat.
2.0 p.m.	131	10	17	12,000	24	3	22	1			Fat.
2.0 p.m.	132	9	16	12,000	24	4	22	1			Salts. Experiment No. 2
2.0 p.m.	133	9	16	12,000	24	4	22	1			Raw Meat.
2.0 p.m.	134	9	16	12,000	24	4	22	1			Raw Meat.
2.0 p.m.	135	9	16	12,000	24	4	22	1			Alcohol.
2.0 p.m.	136	9	16	12,000	24	4	22	1			Alcohol.
2.0 p.m.	137	9	16	12,000	24	4	22	1			Alcohol.
2.0 p.m.	138	9	16	12,000	24	4	22	1			Complete.
2.0 p.m.	139	9	16	12,000	24	4	22	1			Complete.
2.0 p.m.	140	9	16	12,000	24	4	22	1			Garlic.
2.0 p.m.	141	9	16	12,000	24	4	22	1			Garlic.
2.0 p.m.	142	9	16	12,000	24	4	22	1			Sugar.
2.0 p.m.	143	9	16	12,000	24	4	22	1			Sugar.
2.0 p.m.	144	9	16	12,000	24	4	22	1			Sugar.
2.0 p.m.	145	9	16	12,000	24	4	22	1			Alcohol.
2.0 p.m.	146	9	16	12,000	24	4	22	1			Alcohol.
2.0 p.m.	147	9	16	12,000	24	4	22	1			Alcohol.
2.0 p.m.	148	9	16	12,000	24	4	22	1			Meat and Potatoes.
2.0 p.m.	149	9	16	12,000	24	4	22	1			Raw Meat
2.0 p.m.	150	9	16	12,000	24	4	22	1			Raw Meat.
2.0 p.m.	151	9	16	12,000	24	4	22	1			Alcohol.

SWISS MICE: 1 YEAR.

Time of Bleeding.	Experimental No.	Red Blood Count.	Haemoglobin.	White Blood Count.	Lymphocytes.	Monocytes.	Neutrophiles.	Eosinophiles.	Degenerated Cells.	Diet.
			Gm. per 100 cc.							
* 9.48 a.m.	100 K.	10·18	17·5	5,300	31	1	16	2	3	Standard.
9.51 a.m.	100 K.	—	17·5	5,400	—	—	—	—	—	—
9.40 a.m.	121 K.	8·27	17·4	9,430	85	3	10	2	2	Tar Oil.
† —	121 K.	A 9·88	—	3,500	20	2	2	1	2	—
9.30 a.m.	126 K.	9·90	—	10,500	77	12	10	1	4	Meat and Potatoes.
—	126 K.	A 8·03	—	6,600	68	6	24	2	6	—
9.25 a.m.	129 K.	9·05	—	28,400	—	—	—	—	—	Meat and Potatoes.
9.35 a.m.	129 K.	A 8·27	—	9,200	—	—	—	—	—	—
10.25 a.m.	32 K.	11·34	21·52	16,800	63	4	30	3	1	Standard.
†10.20 a.m.	72 K.	9·59	18·2	Aorta 1,500	9	5	8	3	—	Complete.
10.15 a.m.	31 K.	11·1	22·4	22,100	80	2	18	—	3	Sherman.
10.46 a.m.	41 K.	10·46	14·1	25,200	67	4	25	4	10	Sherman + Sugar.
10.00 a.m.	122 K.	9·74	—	13,000	65	8	24	3	—	Sherman + Sugar.
—	122 K.	A 9·77	—	3,600	70	6	21	3	9	—
10.00 a.m.	42 K.	9·37	19·8	17,100	54	11	28	7	3	Meat and Potatoes.
10.05 a.m.	42 K.	9·56	20·0	6,300	—	—	—	—	—	—
10.00 a.m.	48 K.	11·92	21·9	24,300	74	7	18	1	—	Alcohol.
10.03 a.m.	48 K.	—	16·05	16,700	—	—	—	—	—	—
—	—	A —	—	4,200	—	—	—	—	—	—
11.05 a.m.	52 K.	9·92	—	10,500	—	—	—	—	—	Standard.
11.55 a.m.	34 K.	9·5	18·5	10,000	53	5	31	11	5	Complete.
11.10 a.m.	102 K.	9·72	—	11,100	56	8	35	1	2	Complete.
11.13 a.m.	102 K.	—	17·5	9,600	50	6	43	1	—	—
11.05 a.m.	38 K.	10·75	17·5	7,200	59	2	38	1	—	Garlic.
11.15 a.m.	43 K.	10·4	20·7	13,900	76	5	16	3	6	Tar Oil.
11.20 a.m.	43 K.	10·05	19·8	9,350	—	—	—	—	—	—
11.15 a.m.	89 K.	8·29	16·15	10,900	59	4	31	6	4	Tar Oil.
11.18 a.m.	89 K.	—	15·2	10,300	—	—	—	—	—	—
11.30 a.m.	96 K.	9·11	15·7	22,200	82	4	14	—	—	Salts Experiment No. 2.
11.33 a.m.	96 K.	—	—	21,400	—	—	—	—	—	Salts Experiment No. 2.
11.20 a.m.	95 K.	10·79	19·6	20,400	86	1	10	3	—	Alcohol.
11.23 a.m.	95 K.	—	—	21,600	—	—	—	—	—	—
2.20 p.m.	87 K.	10·17	19·3	11,400	72	6	17	5	—	Sherman.
1.35 p.m.	87 K.	9·60	17·8	12,700	63	6	29	2	3	Garlic.
1.38 p.m.	87 K.	—	—	8,500	—	—	—	—	—	Garlic.
1.45 p.m.	93 K.	9·80	20·5	23,100	70	3	26	1	—	Sugar.
1.48 p.m.	93 K.	—	19 1	19,800	—	—	—	—	—	—
1.58 p.m.	94 K.	8·52	18·0	10,500	52	1	47	—	—	Meat and Potatoes.
2.01 p.m.	94 K.	—	17·2	10,500	—	—	—	—	—	Meat and Potatoes.
2.15 p.m.	146 K.	11·33	—	4,300	—	—	—	—	—	Meat and Potatoes.
2.18 p.m.	146 K.	—	—	4,100	—	—	—	—	—	—
2.00 p.m.	40 K.	10·06	15·6	11,950	38	6	38	18	4	Arsenic.
2.40 p.m.	98 K.	10·20	17·5	17,900	78	4	14	4	—	Arsenic.
2.00 p.m.	44 K.	10·52	19·0	13,900	73	2	24	1	1	Salts Experiment No. 2.
2.30 p.m.	45 K.	10·00	20·5	13,350	72	—	25	3	1	Coal Tar.
2.35 p.m.	45 K.	—	18·5	12,100	—	—	—	—	—	—

* Only 50 cells counted. † Only a few cells counted.

SWISS MICE: 2 YEARS.

Time of Bleeding.	Experimental No.	Red Blood Counts.	Haemoglobin.	White Blood Counts.	Lymphocytes.	Monocytes.	Neutrophiles.	Eosinophiles.	Degenerated Cells.	Doubtful.	Diet.
			Gm. per 100 cc.								
9.10 a.m.	125 K.	9. 10	—	10,400	75	21	19	4	3		Standard.
	125 K.				14	7	11	3	8		
		A									
9.35 a.m.	99 K.	9. 25	18.2	19,800	33	8	66	1	1	21	Complete.
10.30 a.m.	51 K.	9. 05	16.8	10,300	87	5	2				Sherman.
10.35 a.m.	51 K.		16.0	9,900	72	6	14	5	1		Meat and Potatoes.
10.45 a.m.	108 K.	9. 69	15.3	39,400							
10.48 a.m.	108 K.			33,800	74	2	20		3		Arsenic.
10.50 a.m.	101 K.	11. 07	20.7	7,000							
			19.4	6,200	74	2	20		3		
11.25 a.m.	46 K.	9. 80	17.0	20,500	73	8	19		1		Standard.
11.30 a.m.	46 K.		20.7	18,700							
2.10 p.m.	103 K.	10. 28		13,200	48	5	35	12			Standard.
2.13 p.m.	103 K.		14.4	12,600							
2.10 p.m.	47 K.	10. 76	18.5	12,600	40	4	51	5	3		Complete.
2.15 p.m.	47 K.			11,500							
2.25 p.m.	104 K.	9. 11		9,000							
2.24 p.m.	104 K.			9,100							Sherman.
2.15 p.m.	49 K.	12. 62	24.7	13,100	82	2.5	11	5	1		+ Meat and Potatoes.
2.23 p.m.	109 K.	9. 19	15.3	18,300	60	3	34	1	1		+ Meat and Potatoes.
2.26 p.m.	109 K.		13.7	14,100							
2.55 p.m.	50 K.	8. 47	16.2	10,300	63	4	26	7	3		+ Arsenic.
2.58 p.m.	50 K.		15.4	8,650							

ROCKEFELLER MICE: 7 MONTHS.

Time of Bleeding.	Experimental No.	Red Blood Counts.	Hæmoglobin.	White Blood Counts.	Lymphocytes.	Monocytes.	Neutrophiles.	Eosinophiles.	Degenerated Cells.	Doubtful.	Diet.
			Gm. per 100 cc.								
10.30 a.m.	139 K.	10·1	20·5	11,500	71	5	14	11	4		Salts Experiment No. 2.
10.33 a.m.	139 K.	—	19·8	8,900							
10.55 a.m.	140 K.	10·23	17·9	15,000	66	6	19	9	1		Alcohol.
10.58 a.m.	140 K.	—	17·5	10,100							
11.30 a.m.	113 K.	10·91	22·1	18,200	80	1	16	3	5		Alcohol.
11.33 a.m.	113 K.	—	—	13,200							
11.10 a.m.	165 K.	10·31	20·3	13,000	78	4	13	5	Many		Alcohol.
11.13 a.m.	165 K.	—	22·1	14,000							
11.20 a.m.	166 K.	9·2	21·7	10,100	73	4	19	4	1		Alcohol.
11.23 a.m.	166 K.	—	18·5	16,900							
12.05 p.m.	155 K.	10·8	21·7	14,100	79	7	14		8		Alcohol.
12.08 p.m.	155 K.	—	—	15,000							
12.00 noon	152 K.	10·23	17·9	11,000	66	6	19	9	1		Tar Oil.
12.03 p.m.	152 K.	—	—	7,500							
1.50 p.m.	115 K.	9·72	17·6	6,000	60		36	4	1		Alcohol.
1.53 p.m.	115 K.	—	15·7	16,400	85	2	12	1			Standard.
1.35 p.m.	114 K.	11·35	20·7	13,800							
1.38 p.m.	114 K.	—	22·7	14,000	70	4	24	2	12		Standard.
1.45 p.m.	167 K.	10·02	19·0	11,000							
1.48 p.m.	167 K.	—	19·4	12,900	71	6	16	5	11		Complete.
1.58 p.m.	168 K.	9·36	18·5	11,000							
2.30 p.m.	168 K.	—	17·4	24,000	63	9	24	4			Alcohol.
2.33 p.m.	132 K.	11·37	17·0	14,800							
2.15 p.m.	132 K.	—	17·9	15,020	72	8	16	4	1		Standard.
2.18 p.m.	131 K.	9·43	17·4	11,300							
2.40 p.m.	131 K.	—	19·1	16,600	58	6	30	4	26		Complete.
2.43 p.m.	156 K.	10·56	20·7	8,500	51	5	35	9	17		Complete.
2.25 p.m.	156 K.	—	19·47	21,900	61	0	25	11	8		Tar Oil.
2.28 p.m.	120 K.	10·81	21·49	14,700							
2.05 p.m.	120 K.	—	20·5	12,600	62	2	30	6			Meat and Potatoes.
2.08 p.m.	119 K.	10·8	21·3	9,100							
2.15 p.m.	119 K.	11·25	19·1	9,200	35	3	6	6	2		Meat and Potatoes.
2.18 p.m.	142 K.	—	18·0	6,200							
2.00 p.m.	142 K.	9·97	21·9	11,300	77	4	19	2	1		Raw Meat.
2.20 p.m.	199 K.	A10·57	—	2,530	39	5	4	3	—		Raw Meat.
2.10 p.m.	200 K.	10·06	21·7	16,100	60	5	18	7	3		Standard.
2.35 p.m.	200 K.	9·95	—	3,700	43	2	21	3	3		Standard.
3.05 p.m.	116 K.	9·74	18·6	11,700	79	2	14	5	11		Salts Experiment No. 2.
3.08 p.m.	116 K.	—	18·6	10,400							Salts Experiment No. 2.

ROCKEFELLER MICE: 1 YEAR.

Time of Bleeding.	Experimental No.	Red Blood Counts.	Haemoglobin.	White Blood Counts.	Lymphocytes.	Monocytes.	Neutrophiles.	Eosinophiles.	Degenerated Cells.	Doubtful.	Diet.
			Gm. per 100 cc.								
9.35 a.m.	161 K.	9 72	20 3	13,100	42	10	42	6	6		Complete.
9.38 a.m.	161 K.		19 3	10,100							
9.43 p.m.	162 K.	10 32	19 6	13,300	74	2	20	4	15		Complete.
9.45 a.m.	162 K.		18 6	17,400							
9.30 a.m.	130 K.	9 64		6,700	152	11	6	4	3		Complete.
9.35 a.m.	163 K.	9 52	17 5	3,500	152	13	11	4	Few		Meat and Potatoes.
10.30 a.m.	35 K.	9 87	19 6	9,400	56	7	33	4	13		Meat and Potatoes.
10.33 a.m.	35 K.	9 42		14,000	70	11	17	2			Complete.
10.21 a.m.	35 K.	9 61	14 9	14,100	67	9	23	1	4		Tar Oil.
10.25 a.m.	35 K.		15 2	14,100	76	14	7	2	1		Tar Oil.
10.30 a.m.	107 K.	9 49	15 9	7,800							Tar Oil.
10.33 a.m.	107 K.		15 1	6,900	91	3	6				Meat and Potatoes.
1.05 a.m.	164 K.	9 1-	19 1	13,700							
1.07 a.m.	164 K.		18 3	10,400	56	4	36	4	1		Complete.
1.20 a.m.	56 K.	9 92	18 4	10,900							Complete.
1.23 a.m.	56 K.		17 6	9,900	55	24	19	26	3		Salts Experiment No. 2.
1.30 a.m.	59 K.	9 67	19 1	12,400	74	10	10	15	15		
1.35 a.m.	154 K.	10 21	20 1	12,100							Alcohol.
1.40 a.m.	154 K.		18 6	10,000	26	15	49	10	3		Alcohol.
12.05 p.m.	53 K.	9 55	15 2	7,300	23	29	33	15	1		
12.07 p.m.	53 K.		16 6	7,400	66	1	25	7	1		Complete.
1.45 p.m.	59 K.	10 44	16 4	13,200							Complete.
1.48 p.m.	59 K.		17 3	10,600	59	9	26	6	7		Meat and Potatoes.
12.55 p.m.	105 K.	10 67	17 3	7,500	67	1	29	3	1		
12.57 p.m.	105 K.	10 12	15 9	15,500							
1.15 p.m.	106 K.	10 63	15 9	15,100	42	6	50	2			Standard.
1.17 p.m.	106 K.		15 6	15,300							Standard.
1.44 p.m.	146 K.	9 16	15 3	17,600	76	1	14	9	1		Standard.
1.46 p.m.	146 K.		14 2	12,400							Complete.
1.10 p.m.	42 K.	7 33	14 2	11,600	42	2	29	1	12		Complete.
1.12 p.m.	42 K.	10 23	16 2		64	7	16				Salts Experiment No. 2.
											Salts Experiment No. 2.
											Alcohol.
											Alcohol.
											Alcohol.

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Studies on the Aetiology of Dunsiekte or Enzootic Liver Disease of Equines in South Africa.

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Dunsiekte, or so-called Enzootic cirrhosis of equines, in South Africa has been carefully studied and described by Theiler (1918). He reviewed the former publications of Robertson (1906) and Verney (1911), and quoted extensively from the work of Gilruth (1902-3) who studied a closely related but not identical disease in equines in New Zealand. Robertson, Verney and Gilruth attributed these liver complaints to certain species of *Senecio*. Theiler (1918) on the other hand, does not support the view that the disease produced by *Senecio latifolius* is identical with Dunsiekte. He maintains that all observations made in the field point directly against the plant as a casual agency. Horses did not eat the plant when it was placed before them or only when it was masked by other foodstuffs. Furthermore, fairly large quantities were necessary to produce any effects and had to be repeated over fairly long periods. Finally horses were not seen to partake of the plant on the pasturage.

The one experiment carried out by him corroborated the common statement by all farmers that ragwort (*Senecio*) was not eaten by horses. However, since ragwort is a plant which appears early at a time when the pasturage is still dry, it was thought that it might be eaten when young. According to Theiler no proof is at hand to show that such was the case. Theiler therefore discards *Senecio latifolius* as a cause of the South African Dunsiekte. He believes that since Gilruth's interpretation of his feeding experiments was to a great extent responsible for the opinion held in South Africa that Dunsiekte was identical with *Senecio* poisoning, closer attention must be given to the facts observed by Gilruth, who accused *Senecio jacobea* as the cause of " Winton disease " in New Zealand. Theiler in analysing the experiments conducted by Gilruth, comes to the conclusion that the suggestion is permissible that the animals died of true ragwort poisoning, but not of a disease identical with Dunsiekte.

Theiler further refers to experiments conducted by himself with *Crotolaria dura* fed to horses and cattle, where cirrhotic lesions were found which resembled somewhat those of Dunsiekte. It would thus appear, according to him, that different plant toxins can produce similar pathological changes. *Crotalaria dura*, however, is not the cause of Dunsiekte, usually it is not found in Dunsiekte areas. Theiler is of the opinion that some other plant must be made responsible and must have the distribution Dunsiekte has, and its ecological habits must stand in some definite relation to the topographic conditions under which the disease occurs. He furthermore comes to the conclusion, from a number of experiments conducted by him, that Dunsiekte is not an inoculable disease, and from the epizootic evidence brought forward the further conclusion is justified that it is not infectious either directly or indirectly. From the above it would seem that Theiler's main objections to regard the *Senecio*, which is abundantly present in the so-called Dunsiekte areas in South Africa, as the cause of Dunsiekte, are the following—

(*a*) that there is a difference in the pathology of Dunsiekte and *Senecio* poisoning mainly as regards the lesions observed in the liver;

(*b*) that the plant is not eaten by horses;

(*c*) that large quantities of *Senecio* are necessary over prolonged periods before *Senecio* poisoning can be brought about.

Theiler was therefore of opinion that some other plant with a distribution corresponding with that of Dunsiekte must, in all probability, be responsible for the disease

Since Theiler's communication, apparently nothing further was published on this subject until quite recently, the results of an investigation of a horse disease in Northwest Nebraska, by van Es and his co-workers (1929) at the Nebraska Agricultural Experimental Station appeared.

"Walking disease" of North-western Nebraska is essentially an enzootic necrobiosis and cirrhosis of the liver due to a toxic agent. The disease affects horses and cattle, but is most commonly encountered in the former. The disease in all its clinical and pathological manifestations may be reproduced with the utmost fidelity by feeding *Senecio riddellii*. It was not possible to exculpate *Senecio integerrimus*, although no experimental evidence was obtained which would indicate that this plant is a factor in the causation of walking disease. There is evidence, epizootologically as well as pathologically, which tends to show that the poisoning by *Senecio riddellii* is limited to a relatively short period. It may be mentioned here that large quanties (48-116-188 lb.) had to be fed for considerable periods before cases were produced. These authors believe that the types of lesions are most likely determined by quantative rather than by qualitative factors. The severest damage is inflicted during the earlier periods of the season, and it is by no means improbable that the very young plants are especially involved in the intoxication. They say that there is some foundation for the belief that in the majority of cases a few pounds of the young leaves may be sufficient to produce a fatal

liver necrobiosis, whereas once the poisonous *Senecio riddellie* plants attain a size large enough to set them apart from the surrounding vegetation, they are no longer eaten by livestock.

Incidentally it may be mentioned that material from natural and experimental cases of " walking disease " of Nebraska was forwarded by Dr. van Es to Onderstepoort to be compared with Dunsiekte in South Africa. The details of this examination will be considered fully by de Kock in a further paper on the pathology of *Senecio* poisoning. For the present it may suffice to state that liver sections from Nebraska cases seem to be identical with those of Dunsiekte cases.

Kimberley disease or " walkabout " disease of North-west Australia, referred to by Murnane and Ewart (1928) is a condition of more or less chronic plant poisoning brought about by continued ingestion of *Atalaya hemiglauca* (and possibly other species of *Atalaya*). The toxic principle must be very slow in its action, and it took them weeks or months to produce fatal effects, even when the plant was taken daily in fairly large quantities. In respect of this disease many interesting points are referred to by Murnane and Ewart. There are stations where *Atalaya* grows, but the disease does not occur, and the authors are of opinion that, if horses are able to obtain abundance of good grass feed, very few will voluntarily eat fresh green *Atalaya*. The writers are inclined to believe that this also takes place with *Senecio* in South Africa, and that the plant is consumed in times of drought in early spring, and especially on those farms where overstocking has been practised. Murnane and Ewart furthermore believe that Winton disease in New Zealand, Dunsiekte in South Africa, and Walkabout disease in North-western Australia, show constant structural liver abnormalities with subsequent cerebral symptoms which are strikingly similar in all three conditions. To these three horse diseases may now be added a fourth, namely, horse disease of Nebraska. Another very interesting statement made about " Walkabout " disease in Australia is that animals developing the disease during the end of the " dry " season show the first symptoms after the commencement of the " wet " season, when food is abundant. It is explained by them that food at that time, on account of its abundance, throws an extra strain and excess of work suddenly on an already diseased liver, which is not capable of functioning normally. Abnormal products results which, in turn, give rise to the final intoxication and disturbance of the central nervous system.

Reference should also be made to the communication of Craig, Kearney and Timoney (1930). They state that as regards the cause of cirrhosis of the liver in horses they have no definite evidence as to its exact nature. They believe it to be some form of vegetable poison and are greatly impressed with the fact that ragwort can set up symptoms and lesions which are similar to those observed in cirrhosis of the liver in horses in Ireland. All the farms on which the disease has occurred are tillage farms which are cropped in ordinary rotation and have an average of 50 acres. There are, however, a few farms where permanent grazing exists. Furthermore, there was one conspicuous feature common to the majority of affected

farms and particularly striking in several instances and that was, that the grazing was of a very poor quality. A low rainfall, or in other words conditions tending towards drought, favours the incidence of the disease. Overstocking and underfeeding also have the greatest possible influence in the production of the disease. On reviewing the different plants observed on surveyed pastures from a toxicological standpoint, they state that one is naturally attracted to the ubiquitous ragwort, in view of its abundance in pastures, in view of its toxic qualities proved by experiments in horses, in view of the lesions it produces in experimental animals, etc.

Jalving (1928) studying the effects of *Senecio jacobea* and *S. aquaticus* poisoning in Holland, believes that it is the cause of enzootic cirrhosis in bovines and that Theiler is not correct in saying that *Senecio* is not eaten by animals at pasture.

In view of this uncertainty as regards the true etiology of South African Dunsiekte, it was decided to commence a number of studies, both at Onderstepoort, where the disease does not occur, and at Kokstad, where Dunsiekte is enzootic and where the mortality from this disease is so high, that horse-breeding has been abandoned on many farms in that area. The studies undertaken deal with various aspects of the problem, e.g. pathological and biochemical considerations, botanical observations on Dunsiekte farms, etc. In view of the careful observations and experiments of Theiler regarding the possibility of Dunsiekte being a communicable disease, more stress was laid on the poisonous plant theory suggested by him. In this paper it is intended to consider certain data relating to the part played by *Senecio* (if any) and, at the same time, to discuss the question whether there are contributory factors to be considered.

A number of experiments was, therefore, planned to elucidate the following points:—

A. *The Question of* Senecio *in all its Various Phases*, viz:—

(1) Its distribution in the Union;

(2) classification of the different species and types;

(3) the toxicity of the various species;

(4) the influence of local conditions on the toxicity of the different species;

(5) stage at which the plant is most toxic;

(6) the influence of overstocking on the preponderance of *Senecio*.

B. *The Susceptibility of the Horse*:—

(1) Whether some horses relish the plant more than others, or whether some animals have depraved appetites;

(2) whether some horses are more susceptible to the toxin of the plant than others.

C. *Contributory Causes*, e.g. climate, veld (pasture), water, etc.

D. *Whether* Senecio *is the Cause of Dunsiekte* and whether the symptoms and pathological changes caused by *Senecio* are identical with those of Dunsiekte.

The greater part of the data relating to botanical, biochemical and pathological studies will be published in subsequent papers. Here it is intended to submit only a preliminary communication about the aetiology of the disease.

Theiler, Gilruth, van Es, and others, definitely admit that *Senecio*, when fed to equines in certain quantities, is toxic and brings about liver disturbances. The point to settle is whether *Senecio* is the cause of such enzootic equine diseases as Dunsiekte, Nebraska Walking disease and Wilton disease. Theiler's difficulties in accepting *Senecio* as the cause of Dunsiekte in South Africa were referred to above. It will, therefore, be advisable to consider in this paper some of the data bearing on this problem.

Senecio is very widely distributed in South Africa. Furthermore, Dunsiekte in equines occurs in those areas where different species of *Senecio* are very prevalent. On the other hand, it must be remembered that there are areas where *Senecio* abounds yet no cases of Dunsiekte have been recorded from there. It must, however, be pointed out that Dunsiekte has been diagnosed chiefly in areas eminently suited to horse-breeding where large numbers of horses are to be found and any undue mortality will immediately be observed. It is, therefore, difficult to maintain with certainty that isolated cases of Dunsiekte did not occur in those areas where fewer horses are kept and where *Senecio* is prevalent. From information on hand, it would appear that the distribution of Dunsiekte is closely related to that of *Senecio*. The areas in Natal, where the disease has most frequently been reported, are the Mooi River, Nottingham Road districts, and the high flat area of the Ixopo district, furthermore Kokstad, in East Griqualand, and Molteno, both in the Cape Province. Sporadic cases are said to occur in the late spring throughout the greater part of the Natal Middleveld area from Karkloof to Estcourt, but these cases are of a more subacute nature than those found in the specific Dunsiekte areas. For that reason a farm, Westlands, in the Mount Currie district was selected for a number of field experiments. This particular farm bears the reputation of being the worst Dunsiekte farm in the district. It is situated about five miles from Kokstad on the main road to Umtata. Probably due to overstocking, Westlands was found to be overgrown with different species of *Senecio*, in fact in portions of camp A it almost represented a sown field. Apart from everything else, it is difficult to conceive how horses can graze on these portions without daily consuming a certain amount of *Senecio*, more especially when the plants are young and succulent and the grass more or less of the same height. In fact, in the early spring horses were carefully watched during their grazing and a fair amount of the plant was actually seen to be consumed.

Unfortunately our knowledge of the different species of *Senecio* in South Africa and the extent to which they occur in different parts of the Union, is very fragmentary. Great difficulty is experienced in the classification of the different species and types and for the present

the *Senecios* collected at Westlands have been designated by numbers. The following is a brief description of the various types or species found in the four camps, A, B, C, and D, at Westlands.

Variety 1.

Mountain Species: Lanceolate leaves, edges serrated. Texture of leaves very harsh and dryish, darkgreen with an ashy sheen.

Flowering Heads: Multiple; yellow; petalled; coarse. Stalk very tall and succulent (eaten by animals).

Habitat: Hill tops growing amongst boulders in clumps or slightly scattered over confined areas, usually more or less in sheltered spots.

Occurrence: Found, not plentifully, in Camp A.

Variety 2.

Cabbage Species: Spatulate leaves, edges smooth but somewhat curly; colour dark green; very pungent odour; luscious and luxuriant growth.

Flowering Heads: Multiple; yellowish; fine; very tall stalk, practically denuded of leaves from height just above egress from plants.

Habitat: Edges of newly ploughed field or soft low lying areas, i.e. water courses and vleis.

Occurrence: Common in all camps.

Variety 3.

Harsh Leafed Species: Leaves spatulate with sharp tip; very hard and tough; colour greyish-green.

Flowering Heads: Multiple large (3 to 5 on each flowering head) yellow; petalled; stalk not more than 6-9 in.

Habitat: Flats or slight slopes, growing singly.

Occurrence: Found in camps A and D, and homestead.

Variety 4.

Second Common Variety: Foliage very fine and lanceolate. Colour dark green.

Flowering Heads: Luxuriant growth; multiple coarse flowers, thistle shaped; yellow; stalks about 12 in. to 16 in. in height.

Habitat: Slopes, especially hollows and sheltered places thereon.

Occurrence: Abundant where present. Found only in two places in Camp A, and on the slope below homestead.

Variety 5.

Large Semi-spatulate Variety: Tips of leaves pointed; lighter green in colour.

Flowering Heads: Multiple; fine, closely resembling Variety 2; yellow; stalks very tall reaching height of 2 ft. to 2 ft. 6 in., leaves in decreasing size present all the way up.

Habitat: Water courses.

Occurrence: Plentiful. Found in all furrows running through camps.

Variety 6.

Common (latifolius) Species: More or less scraggy plant. Leaves darkish green, fine (but coarser than Variety 4).

Flowering Heads: Multiple; flowers petalled; yellow; stalks up to 18 in. high.

Habitat: Flats and slopes.

Occurrence: Profusely distributed over whole farm

Variety 7.

Furrow Lanceolate Species: Leaves lightish green, very narrow and long.

Flowering Heads: Multiple; fine, closely resembling Varieties 2 and 5; stalks very tall, up to 3 in. high.

Habitat: Water courses only.

Occurrence: Fairly abundant and found in most furrows on farm.

Variety 8.

Shrubby Species: Foliage very fine and dark green. Plant very rank and takes on a form peculiar to its environment, e.g. on level ground as shrub, in depressions or on banks thereof as recumbent spreading growth. Shrubs about 9 in. to 12 in. high.

Flowers: Single; yellow; petalled.

Habitat: General distribution. Found in holes in veld, on flats especially near and on the edges of paths, in abandoned lands (grows very profusely) and along fences, which would lead to the belief that seeding occurs through birds.

Occurrence: Very plentiful over whole farm.

It may be mentioned that in Camp A there was "Mountain grazing" chiefly and *Senecio* was very plentiful; in patches it resembled sown fields of *Senecio*. In this camp, as will be shown later, the mortality in the field experiments, was the highest.

In Camp B the pasture was "old lands" with a good deal of *Senecio* present, and this camp was believed to be responsible for a number of deaths.

In Camp C there was "vlei" grazing and in Camp D "natural grazing." These types were also believed to be responsible for a number of deaths. All four camps were utilized for the experiments to be considered later on.

The question of the toxicity of the different species of *Senecio*, the influence of local conditions on the toxicity and the stage at which the plant is most toxic, is still under consideration, and will be discussed in subsequent papers. Suffice it to say that some farmers are of the opinion that the young plant is more toxic, but this opinion is probably based on the fact that the young plant is more liable to be eaten accidentally with the short grass. It would appear that the disease is seasonal; that may be due either to the fact that the plant is more toxic in some years (c.f. Gousiekte caused by *Vangueria pygmea*), or that the grazing, especially in spring, is decidedly better in some years and therefore less of the plant is consumed

A Consideration of the Various Experiments Conducted at Kokstad and at Onderstepoort.

These may, for convenience, be divided into the following groups:—

I. Field experiments at Kokstad.

II. Drenching experiments:

(*a*) at Kokstad;

(*b*) at Onderstepoort.

1. *Field Experiments.*

As previously stated the farm Westlands in the Mount Currie District, with an extremely bad Dunsiekte reputation, was utilized for the experiments which were started on the 29th November, 1928, and continued up to 7th January, 1931, when the remaining control horses in these experiments were transferred to Onderstepoort. The horses utilized for these experiments at Kokstad had been in various horse-sickness immunization experiments and were previously bought from dealers for this purpose. In the majority of cases they were cast horses which came from various parts of the Union and it is impossible to say whether a few had not been bred in Dunsiekte areas. When they left Onderstepoort for Kokstad, they were immune against horse-sickness, in good condition, and their ages varied from 5 years to 10 years and over. In all, 34 horses were utilized, some of these arrived at Kokstad six months after the first batch.

Westlands is divided into four camps, A, B, C, and D, and into each of these a certain number of horses was drafted (see Tables I and II). During the winter of 1929 and 1930 the grazing was so poor that the majority of the horses in the various grazing experiments lost in condition, and in some cases extra food had to be given.

TABLE I.

Dunsiekte Experiments at Kokstad.

D.O.B. No.	Experiment Commenced.	Treatment.	Remarks.	Path. Spec. No.	Pathological Diagnosis.
18932....	19/12/28	Muzzled* in Camp A.....	No symptoms, slipped muzzle several times, killed 27/12/30	11096	Very slight reparative, regressive and proliferative changes.
18949....	10/1/29	Tethered† on Senecio in Camp A	No symptoms, killed 27/12/30.....	11098	Nothing unusual.
18964....	29/11/28	Grazing and dipped. Camp A...	Definite symptoms of Dunsiekte, killed 27/12/30..	11101	Dunsiekte. Note characteristic proliferations and bile duct epithelium.
18956....	29/11/28	Grazing, dipped and treated with CS_2. Camp A	Definite symptoms of Dunsiekte, died 29/11/30...	10997	Slight reparative, regressive and proliferative changes.
18997....	29/11/28	Grazing. Camp A.....	Symptoms, killed *in extremis* 22/12/30.....	11079	Dunsiekte. Marked proliferation of bile duct epithelium.
19018....	24/12/28	25/8/30 Tethered †on Senecio area, Camp A	No symptoms, killed 27/12/30.....	11108	Very slight reparative changes.
19024....	4/5/29	Grazing, Camp A.....	No symptoms. 7/1/31 returned to Onderstepoort and in excellent condition on 5/3/31.		
19183....	4/5/29	Grazing, Camp A.....	Slight symptoms but recovered; 7/1/31 returned to Onderstepoort in excellent condition. Killed 7/3/31.	11323	Reparative changes, slight cirrhosis and proliferation of bile ducts, haemosiderosis in stern cells.
19199....	4/5/29	Grazing, Camp A.....	Slight symptoms. 7/1/31 returned to Onderstepoort and killed 22/1/31	11196	Slight reparative, regressive, and proliferative changes.
18788....	4/5/29	Grazing, Camp C and Camp B..	No symptoms. 7/1/31 returned to Onderstepoort. Transferred to Senecio drenching experiment.		
18945....	8/1/29	Tethered† Senecio-free area and dipped	Killed 28/12/30.....	11097	Very slight regressive and reparative changes
18951....	17/1/29	Muzzled*, Camp B.....	No symptoms. Killed 27/12/30.....	11100	Slight reparative and proliferative changes.
18967....	29/11/28	Grazing, Camp B and dipped....	No symptoms, transferred to Onderstepoort 7/1/31		
19005....	29/11/28	Grazing and dipped, Camp B....	Very slight symptoms. Killed 27/12/30.....	11106	Very slight reparative changes.
19106....	4/5/29	Grazing, Camp C and Camp B..	No symptoms. 7/1/31 returned to Onderstepoort in excellent condition on 5/3/31		
19201....	4/5/29	Grazing, Camp C. Grazing, Camp B.	Very slight symptoms. Killed 28/12/30.....	11109	Nothing unusual.
19401....	4/5/29	Grazing, Camp B.....	Very slight symptoms. Killed 27/12/30.....	11110	Nothing unusual.
18948....	29/11/28	Grazing, Camp B.....	No symptoms. Killed 25/5/29.....	9281	Nothing unusual.
19173....	4/5/29	Grazing, Camp B.....	Slight symptoms. Died 27/3/30.....	10164	Chronic case of Dunsiekte.
18688....	4/5/29	Grazing, Camp B.....	Symptoms. Killed 18/11/29.....	9801	Acute case of Dunsiekte (circulatory).
18953....	29/11/28	Grazing, Camp C, dipped and treated with CS_2	No symptoms. Killed 27/12/30.....	11099	Slight proliferative changes.

NOTE.—* As explained on page 629 the muzzling experiment was not a success, as the muzzled horses on several occasions lost their muzzles and grazed with the rest of the animals. The only reason why the muzzling and tethering experiments are referred to in this table, is to emphasize the fact that the horses in these experiments were restricted in their grazing for most of the time.

† The remarks in footnote * refer also to the tethering experiment. These horses on various occasions broke loose and grazed with the other animals.

TABLE I—(*continued*).

D.O.B. No.	Experiment Commenced.	Treatment.	Remarks.	Path. Spec. No.	Pathological Diagnosis.
18959....	29/11/28	Grazing, Camp C.....	Very slight symptoms. 7/1/31 returned to Onderstepoort in good condition on 5/3/31		
18969....	4/5/29	Grazing, Camp D and Camp C..	Very slight symptoms. 7/1/31 returned to Onderstepoort, transferred to Senecio drenching experiment		
18980....	29/1/29	Grazing, Camp D and Camp C..	,, ,, ,, ,,		
18981....	17/1/29	Muzzled*, Camp C.....	No symptoms. Killed 27/12/30.....	11103	Slight lesions of Dunsiekte.
18982....	17/1/29	Tethered†, Senecio-free area.....	Killed 28/12/30.....	11104	Slight reparative changes.
18999....	11/12/29	Grazing, Camp D and Camp C...	Very slight symptoms. Killed at Onderstepoort 16/1/31	11171	Slight reparative changes.
19015....	27/11/28	Grazing and dipped, Camp C....	Very slight symptoms. Killed 28/12/30.....	11107	Very slight reparative changes.
18883....	19/12/28	Tethered† Senecio-free, Camps B and D. Dipped	Killed 28/12/30.....	11095	Very slight reparative changes.
18968....	2/12/28	Grazing and dipped, Camp D...	Very slight symptoms. Killed 28/12/30.....	11102	Very slight reparative changes.
18996....	4/12/28	Grazing, Camp D.....	No symptoms. Returned to Onderstepoort 7/1/31. Killed 16/1/31	11172	Very slight reparative changes.
19003....	17/1/29	Muzzled*, Camps C and D.....	Very slight symptoms. Killed 28/12/30.....	11105	Nothing unusual.
19292....	4/5/29	Grazing, Camps B and D.....	No symptoms. Returned to Onderstepoort 7/1/31, in excellent condition on 5/3/31		
19009....	4/5/29	Grazing, Camp D.....	Symptoms, Died 26/8/30.....	10670	Acute Dunsiekte (circulatory).
19176....	4/5/29	Grazing, Camp C, 20/8/30 to Transport	No symptoms. 7/1/31 returned to Onderstepoort in excellent condition on 5/3/31		
19177....	4/5/29	,, ,,	,, ,, ,, ,,		
19595....	4/5/29	,, ,, 18/9/30 to Transport	,, ,, ,, ,,		

NOTE.—* As explained on page 629 the muzzling experiment was not a success, as the muzzled horses on several occasions lost their muzzles and grazed with the rest of the animals. The only reason why the muzzling and tethering experiments are referred to in this table, is to emphasize the fact that the horses in these experiments we restricted in their grazing for most of the time.

† The remarks in footnote * refer also to the tethering experiment. There horses on various occasions broke loose and grazed with the other animals.

TABLE II.

Summary of Dunsiekte Experiments at Kokstad.

D.O.B. No. of Horses.	*Grazing.*	Killed.	Died.	Arrival to 1st symptoms.	Arrival to death.	*Tethered on Senecio area.*	Killed.	Arrival to death.	*Tethered Senecio-free area and dipping.*	Killed.	Arrival to 1st symptoms.	Arrival to death.	*Tethered Senecio-free area.*	Killed.	Arrival to 1st symptoms.	Arrival to death.	*Muzzled.*	Killed.	Arrival to death.	*Grazing + dipping.*	Killed.	Arrival to 1st symptoms.	Arrival to death.	*Grazing + dipping + CS_2.*	Killed.	Died.	Arrival to 1st symptoms.	Arrival to death.
Camp "A."				Days.	Days.			Days.			Days.	Days.			Days.	Days.			Days.			Days.	Days.				Days.	Days.
19018.....	—	—	—	—	—	—	—	—	—	—	—	—	1	1C	—	733	—	—	—	—	—	—	—	—	—	—	—	—
19024.....	1	—	—	—	—	Still	livi	ng and	d in	exc	ellent	cond	ition.				—	—	—	—	—	—	—	—	—	—	—	—
19199.....	1	1C	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
18997.....	1	1D	—	752	754	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
18956.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
18964.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	1D	708	730
18932.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1D	742	757	—	—	—	—	—
18949.....	—	—	—	—	—	1	1N	718	—	—	—	—	—	—	—	—	1	1C	757	—	—	—	—	—	—	—	—	—
19183.....	1	1C	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Camp "B."																												
18945.....	—	—	—	—	—	—	—	—	1	1C	—	719	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
18948.....	1	1N	—	—	177	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
19173.....	1	—	1D	286	327	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
18688.....	1	1D	—	196	198	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
18951.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
19005.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1C	709	—	—	—	—	—	—	—	—	—
19201.....	1	1N	—	484	603	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1C	550	758	—	—	—	—	—
19401.....	1	1N	—	133	603	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
18788.....	1	—	—	—	—	Still	livi	ng and	d in	exc	ellent	cond	ition.				—	—	—	—	—	—	—	—	—	—	—	—
18967.....	1	—	—	—	—	—	—	—	—	,,							—	—	—	—	—	—	—	—	—	—	—	—
19106.....	1	—	—	—	—	—	—	—	—	,,							—	—	—	—	—	—	—	—	—	—	—	—

TABLE 11—(*continued*).

D.O.B. No. of Horses.	*Grazing.*	Killed.	Died.	Arrival to 1st symptoms.	Arrival to death.	*Tethered on Senecio area.*	Killed.	Arrival to death.	*Tethered Senecio-free area and dipping.*	Killed.	Arrival to 1st symptoms.	Arrival to death.	*Tethered Senecio-free area.*	Killed.	Arrival to 1st symptoms.	Arrival to death.	*Muzzled.*	Killed.	Arrival to death.	*Grazing + dipping.*	Killed.	Arrival to 1st symptoms.	Arrival to death.	*Grazing + dipping + CS_2.*	Killed.	Died.	Arrival to 1st symptoms.	Arrival to death.
Camp "C."				Days.	Days.	Still	living and in	Days.	Still	living and in	Days.	Days.	Still	living and in	Days.	Days.			Days.			Days.	Days.				Days.	Days.
1890	1																											
1893																												
1894																												
1895	1																											
1899	1	1C																										
1909	1																											
1915																												
Camp "D."																												
1896	1	1C			630	Still	living and in	Days.	Still	living and in	Days.	Days.	Still	living and in	Days.	Days.			Days.			Days.	Days.				Days.	Days.
19009	1		1D	554																								
19292	1																											
1883																												
1908																												
19003																												

Abbreviations D.—Dunsiekte.
C.—Liver changes when subsequently killed.
N.—No liver changes.

The original plan was to conduct the following five experiments on the farm Westlands:—

I. *Grazing Experiment in Camps A, B, C, and D.*—The horses were to run in these camps day and night; the object of the experiment being to ascertain the prevalence of Dunsiekte in the various camps during the successive seasons of the year.

II. *Grazing and Dipping Experiment.*—Horses were to be dipped regularly at weekly or fortnightly intervals in order to determine whether dipping has any preventive effect on the incidence of Dunsiekte, as is commonly believed by farmers.

III. *Grazing, Dipping and Treatment with Carbon Bisulphide.*—The treatment with CS_2 was included in this experiment to test the theory held by farmers and others that *Gastrophilus* larvae (" bots ") played a rôle in the causation of Dunsiekte and that the disease could be checked if these larvae were destroyed by means of CS_2.

IV. *Muzzling Experiment.*—The horses were to be muzzled and allowed to run with the other animals during the day, whereas they were to be stabled and fed at night. The object of the experiment was to ascertain whether there was some factor not connected with the veld, responsible for Dunsiekte.

V. *Tethering Experiment.*—Some horses were to be tethered on plots free from *Senecio*, and others on plots where *Senecio* was present. Fresh plots were to be selected from time to time. The object, of course, was to get more direct evidence on the aetiological rôle of *Senecio*.

These experiments were actually started, but it soon became apparent that it was impossible to carry out the entire programme satisfactorily. The chief difficulty was the great distance of the farm from Pretoria and the consequent impossibility of maintaining adequate supervision over the experiments. It was found, after a time, that some of the muzzled horses had, on occasions, lost their muzzles, and that some of the tethered animals had broken loose and grazed with the rest. Consequently it was impossible to draw any conclusions from these particular experiments.

Nevertheless, valuable data were collected in the course of the Kokstad experiments.

Many of the horses killed at the termination of the experiment revealed slight liver lesions microscopically, although these animals during life had never shown symptoms of Dunsiekte. These lesions will be discussed fully by de Kock in a subsequent paper on the pathology of the disease. Suffice it to say here that these lesions were chiefly of the nature of reparative changes, i.e. that the liver

at some time or other had been damaged and healing was taking place, in some instances associated with slight cirrhosis and proliferation of bile duct epithelium. Unfortunately these important results only became evident, when the experiment had been closed down. In case of Horse 19183 very slight symptoms of Dunsiekte, e.g. dullness, listlessness, staring coat, were reported on the 8-11th September, 1930. These symptoms were, however, of such a slight nature that they could easily have escaped the notice of the assistant in charge if a careful daily examination of the horses had not been carried out. Such a careful examination was, however, not always possible in these large camps. This Horse 19183, since its return to Onderstepoort on 7.1.31 certainly showed no symptoms and was in very good condition when killed on 7.3.31. Macroscopically the liver lobulation was rather prominent and microscopically definite lesions were identified, e.g. slight cirrhosis, slight proliferation of bile duct epithelium, and evidence of an earlier damage to the liver parenchyma, indicated by the derangement of the columns of liver cells, and reparative changes. This horse, and the majority of the other controls from Kokstad, showed liver lesions, in spite of the fact that they were clinically healthy when they were destroyed. It would appear, therefore, that in many horses in a Dunsiekte area no clinical symptoms become manifest because insufficient damage to liver parenchyma had taken place. There are undoubtedly many such cases which escape the notice of farmers and horse dealers, and it may be just these cases which, after some time (6 months and longer) succumb to Dunsiekte in a non-Dunsiekte area. This type of case naturally interferes markedly with the interpretation of the experiments and it becomes, therefore, essential that all horses at the expiration of the experiment should be destroyed to study the liver changes, unless some biochemical means can be evolved for the detection of very slight liver disturbances in such cases. Furthermore, on account of these obscure cases, it is almost impossible to give an accurate idea of the number of animals affected naturally, in the different experiments. Moreover, it would appear that the figures for cases of Dunsiekte given in Table II, should really be higher. On the other hand, it should be pointed out that horses which had revealed slight symptoms of Dunsiekte (e.g. Horses 19201 and 19401) showed no liver changes when destroyed some time afterwards. This would indicate that horses may show symptoms attributed to liver lesions and yet pathologically make a complete and fairly rapid recovery, i.e. " restitio ad integrum."

From the field experiments it may be concluded definitely that dipping and dosing of horses with carbon bisulphide did not check Dunsiekte. It may also be mentioned that the regular dosing with carbon bisulphide freed horses completely from *Gastrophilus* larvae but did not prevent the disease. Furthermore, when referring to Tables II and III it will be seen that during the two years the mortality from Dunsiekte on the farm Westlands was very high. Out of 25 horses (i.e. grazing and dipped) there were 6 cases of Dunsiekte, i.e. a percentage mortality of 24 and that the greatest percentage of cases occurred in Camp A where *Senecio* was the most abundant.

TABLE III.

Summary of the Number of Cases of Dunsiekte in the Various Camps.

No. of Camp.	No. of horses in the Camp.	No. of cases of Dunsiekte.	No. of horses killed and liver found to be normal.	No. of horses killed and liver found to show liver lesions.	No. of animals originally in the Camp still living at Onderstepoort.
A.....	9	3	1	4	1
B.....	11	2	3	3	3
C.....	8	1*	—	4	3
D.....	6	1	1	3	1

* Muzzled horse which never showed symptoms, yet when it was destroyed, showed lesions of Dunsiekte which, however, were not pronounced.

The earliest natural case occurred 196 days, and the latest 752 days after the arrival of the animals on a farm with a Dunsiekte reputation.

Furthermore, it is interesting to note that in two cases death occurred or the animal was killed *in extremis* a few days after the onset of symptoms. This period is usually much longer. Another point of interest is the fact that all the horses at Kokstad with and without liver lesions showed the presence of abnormal quantities of a straw coloured fluid in the abdominal cavity (e.g. Horse 19003 which showed no liver changes).

Variations in Susceptibility have been reported, but there are no definite facts to go on. It would appear that the number of cases which have been noted in horses of seven years and over has been small. Most of the cases are in young horses of 3, 4 and 5 years. It must be pointed out, however, that all the natural cases of Dunsiekte at Westlands (25 per cent.) were in animals over 6 years up to 12 years and even older. In a very susceptible animal it is said the period of exposure need not be very long. On the other hand, clinical evidence of the disease may not be seen for a period of about two years from last contact with a Dunsiekte area. There are definite records of cases which occurred in the Durban Municipal stables, among horses purchased at three years old, bred in the Nottingham Road area and dying of the disease two years later. (Vide Report No. 46 of the 4th June, 1924).

II. *Drenching Experiments.*

(*a*) At Kokstad (i.e. in a Dunsiekte area).

(*b*) At Onderstepoort (i.e. in an area where Dunsiekte is not known to occur.

A brief summary of the various drenching experiments carried out with *Senecio* is given in Table IV.

TABLE IV.

A. *Senecio Drenching Experiments at Kokstad, etc.*

D.O.B. No. of Horse.	Experiment Commenced.	Amount of Senecio fed. lb.	No. of Days Fed.	No. of Days up to First Symptoms.	Treatment.	Remarks.	Path. Spec. No.	Pathological diagnosis of Changes in the liver.
19687..	9/10/30	—	—	—	Fed on Malta Thistle...	No symptoms. Killed, Kokstad 28/12/30	11111	Nothing unusual.
19396..	20/2/30	137	76	About 60.....	Drenched fresh Senecio 1–2·6 lb. twice weekly	Chronic case of Senecio poisoning. Died 25/11/30	10988	Circulatory and proliferative changes, also reparative.
19462..	20/2/30	92·5	54	About 45.....	Drenched 1–4 lb. fresh Senecio twice weekly	Chronic case of Senecio poisoning. Died 29/8/30	10671	Slight reparative, regressive and proliferative changes.
19168..	4/5/29	46·5	31	About 60.....	Drenched ½ lb. fresh Senecio twice weekly	Chronic case of Senecio poisoning. Died 2/2/30	9985	Slight reparative, regressive and proliferative changes.
18947..	30/1/29	54·0	36	About 75.....	Drenched ½ lb. fresh Senecio twice weekly	Chronic case of Senecio poisoning. Killed at Onderstepoort 30/10/29	9774	Resembles chronic Dunsiekte.
19006..	5/12/28	18·5	37	About 14.....	Drenched ½ lb. fresh Senecio twice weekly	Returned to Onderstepoort 13/6/29. Killed at Onderstepoort 24/7/29	9474	Circulatory changes and cirrhosis.
19027..	4/5/29	89·8	33	About 38.....	Drenched 1·2 Kg. fresh Senecio twice weekly	Died 19/10/30.....	10844	Resembles chronic Dunsiekte with extensive cirrhosis.
16007..	20/11/25	86	About 75	About 75.....	Fed on Senecio at Pietermaritzburg	Symptoms resemble Dunsiekte. Died 17/2/26	5875	Acute circulatory changes like Dunsiekte.

B. *Senecio Drenching Experiments at Onderstepoort.*

19226..	18/11/29	5·7	308	135	Fed variable quantities 0·01–0·125 lb. daily	Yawning, icterus, apathy, and finally acute staggers. Killed 7/10/30	10772	Resembles chronic case of Dunsiekte with reparative changes.
19253..	6/11/28	2·4	12	4	Drenched variable quantities *S. isatidius*	Icterus, apathy inappetence. Died 20/11/28	8638	Resembles early case of Dunsiekte, i.e. circulatory and regressive changes.
19643..	17/9/30	1·6	6	2	·25 lb. dry Senecio from Kokstad daily fed with food	do. and bleeding from nostril. Died 24/9/30	10736	Circulatory and regressive changes, slight cirrhosis.
19657..	17/9/30	8	30	27	Variable quantities of Senecio from Kokstad eaten daily	do. Killed 17/10/30.....	10812	do. slight reparative changes.
18824..	5/1/31	2·5	10	3	·25 lb. of dry Senecio, Camp A, Kokstad drenched daily.	Apathy, icterus, etc., symptoms of staggers. Killed 16/1/31	11174	Circulatory changes and regressive changes.
19588..	5/1/31	3·25	13	3	do. Camp B.....	Apathy, icterus, yawning. Died 20/1/31	11180	Circulatory (lagoons), changes, regressive changes, proliferation of bile duct epithelium.
19664..	5/1/31	2·75	11	3	do. Camp C.....	do. Killed 16/1/31.....	11173	Haemosiderosis and slight cirrhosis.

TABLE IV—(*continued*).

D.O.B. No. of Horse.	Experiment Commenced.	Amount of Senecio fed. lb.	No. of Days Fed.	No. of Days up to First Symptoms.	Treatment.	Remarks.	Path. Spec. No.	Pathological diagnosis of Changes in the liver.
19590..	5/1/31	4·25	17	16	·25 lb. of dry Senecio, Camp D, Kokstad drenched daily	Apathy, ieterus, yawning. Killed 25/1/31	11200	Circulatory, regressive, and slight proliferative changes (Bile duct epithelium).
18470..	20/1/31	3	12	10	·25 lb. dried Senecio Kokstad drenched daily	do. and symptoms of staggers. Died 1/3/31	11303	Circulatory (lagoons) changes and slight cirrhosis.
19299..	20/1/31	5·75	23	14	do. do.	do. Killed 15/2/31.....	11275	Circulatory (extensive) and regressive changes; slight cirrhosis.
19068..	21/1/31	5·25	21	—	do. Camp D.....	No symptoms. Killed 7/3/31.....	11322	Slight cirrhosis, proliferation of bile ducts, pigmentation in periphery.
18788..	27/1/31	3	12	7	do. Camp B. Horse from Kokstad	Symptoms of acute staggers. Killed 10/2/31	11255	Extensive circulatory, regressive reparative changes and cirrhosis and exudative changes.
18980..	27/1/31	1·25	5	3	do. Camp C.....	Symptoms of staggers, bleeding from nose, anus, eyes. Killed 2/2/31	11237	Circulatory (extensive) and regressive changes, slight cirrhosis.
18461..	20/1/31	·25	1	—	Drenched Senecio from Kokstad	No symptoms, control. Killed 21/1/31..	11188	Nothing unusual.
20039..	11/2/31	10 oz.	1	—	Drenched dry Senecio Kokstad	No symptoms, control. Killed 16/2/31..	11276	Nothing unusual.

At Kokstad fresh *Senecio* plants were used, whereas at Onderstepoort *Senecio* collected and dried at Kokstad was utilized. In the majority of the experiments the *Senecio* plants were minced and drenched in the form of a suspension through a stomach tube. Besides occasional slight bleeding from the nose, no bad results were evidenced by the regular daily use of the tube.

It should, however, be pointed out that cases of *Senecio* poisoning were not only brought about by drenching the dry plant suspended in three litres of tapwater, but also by means of balling with the moistened ground plant. We realize that the lethal or toxic dose can only be determined accurately when the toxic principle of the plant is known. These experiments are still proceeding, but it may be mentioned here that cases of *Senecio* poisoning were brought about more consistently and quickly when plants in the pre-flowering stage were utilized.

If Table IV is scrutinised, it will be seen that an acute fatal form of poisoning could be produced by drenching. In one case only $1\frac{1}{4}$ lb. of dried *Senecio* were sufficient to cause symptoms to appear 3 days after the commencement of drenching and death in 6 days. In the Onderstepoort experiments, where daily drenching was resorted to, the total quantities of *Senecio* drenched before death occurred, with the exception of two cases (viz. Horses 19657 and 19068), never exceeded 6 lb. At Kokstad much larger quantities (from 18.5 lb.—92.5 lb.) of the fresh plant had to be drenched before symptoms occurred. Note the following cases at Kokstad:—

Horse 19396 showed first symptoms after 266 days.
Horse 19462 showed first symptoms after 160 days.
Horse 18947 showed first symptoms after 273 days.
Horse 19168 showed first symptoms after 274 days.
Horse 19027 showed first symptoms after 168 days.
Horse 19006 showed first symptoms after 230 days.

How is this rather significant difference between the Kokstad and Onderstepoort experiments to be explained? Is it here perhaps a question of the *Senecio* poison being given daily in a more concentrated form? At Kokstad the fresh plant was fed, whereas the non-volatile poison was more concentrated in the dried plant after the loss of water, i.e. the dried plant fed at Onderstepoort lb. for lb. contained much more *Senecio* poison. Daily doses of 120 grams of dried *Senecio* were used as a basis at Onderstepoort and symptoms appeared from 2-16 days after drenching was commenced. It should, however, be remembered that *Senecio* from different camps of different growth, were indiscriminately used at first. Comparative tests in this respect and with different species of Senecio are still in progress.

The pathology of all these cases will be fully considered by de Kock in a subsequent paper, suffice it to say here that the characteristic lagoons of blood in the liver, described by Theiler in the initial

stages of Dunsiekte, were very prominent in Horses 16007 and 18470, etc. of the Onderstepoort drenching experiments. Besides these circulatory, regressive, reparative and proliferative changes of connective tissue and bile duct epithelium, there was no evidence, except in one case (Horse 18788) of a parenchymatous hepatitis. In some of the very acute cases of *Senecio* poisoning extensive haemorrhages were observed in the nasal cavity, conjunctiva, peritonium, pleural and extensively so in the intestines, especially in the small intestine and also in the bladder. It would, therefore, seem that *Senecio* poisoning or Dunsiekte in the initial stage is a disease of the circulatory system. This question is still under consideration. On the other hand, amongst the drenched animals at Kokstad cases occurred which resembled the later stage of Dunsiekte described by Theiler.

A study of the blood was made on the following horses drenched with *Senecio* at Onderstepoort, viz.: 19664, 19590, 18470, 19068, 18788 and 18980, from the time the experiment was commenced until the horses died or were killed. As regards the erythrocytic count, red precipitate, total leucocytic count and differential leucocytic count, no abnormalities or changes were observed. This is rather interesting because in acute liver atrophy of the horses, de Kock (results still to be published) observed interesting changes in a batch of horses (in February and March, 1929) where the mortality was about 50 per cent. He was actually able to pick out cases (Horses 19343 and 19335) before symptoms of acute liver atrophy occurred, by blood studies of all the remaining horses in this batch after the first few cases had occurred. The characteristic changes were an increased erythrocytic count 11-13 millions erythrocytes per c.mm., a definite bilirubinaemia evidenced in the supernatant serum of the precipitate test. This was attributed to the extensive damage of the liver parenchyma in acute liver atrophy. It was not classified as a polyglobuli, but as a condensation of the blood. In *Senecio* poisoning where extensive liver changes occurred no such changes in the blood were identified. The study of the plasma and serum of the blood of these animals in the *Senecio* drenching experiments, especially in respect of bile pigments and Amino acids (to be fully discussed in a subsequent biochemical paper), was undertaken to ascertain whether some method of diagnosis could not be evolved to detect those cases with slight liver changes.

The symptoms of *Senecio* poisoning were those associated with liver disturbance, and varied according to the degree of destruction of liver parenchyma. These have been fully described by Gilruth, van Es, Theiler and others, and need not be referred to again. Suffice it to say that cases of *Senecio* poisoning were produced which resembled the symptoms of the initial and later stages of Dunsiekte described by Theiler. In a few cases symptoms of mad staggers appeared shortly before death.

In this respect a full consideration of the symptoms, etc., observed in Horse 19226 (6 months old) and Horse 18969 fed with dried *Senecio* will be of interest.

Horse 19226.—In the course of ten months this animal had received 2,740 grams of dried *Senecio isatidens* and 810 grams of dried *Senecio latifolius* (recently named *S. retrorsus*).

Result: The first disturbances were noticed on 3.4.30, that is, four and a half months after the inception of the experiment. Up to this date the animal had ingested 1,120 grams of *Senecio isatideus*. The symptoms exhibited were slight inappetence, drowsiness, conjunctivae reddish in colour, vessels markedly injected, respiration laboured and of the abdominal type. The pulse was normal. These symptoms slowly increased in intensity until on 16.6.30 there were complete inappetence, an injected conjunctiva with a light yellowish tinge, an accelerated pulse (80 per minute), laboured respiration, fibrillary contractions of the muscles of the hind legs and tail and continued yawning. There was progressive loss in condition and the animal appeared tired, resting the hind legs alternately at short intervals. A few days later it was seen standing with half closed eyes and swaying from side to side.

On 26.6.30 the feeding of the plant had to be discontinued for a month on account of lack of material. There was a marked improvement in the animal's condition within seven days after cessation of the feeding of the plant. The improvement progressed until the animal appeared quite normal on 25.7.30 when the feeding with dried *Senecio latifolius* commenced.

The 300 grams of *Senecio latifolius* fed from 25.6.30 to 29.7.30 caused a slight precipitation of the previous symptoms. Feeding had again to be discontinued from 3.7.30 to 3.9.30 until further supplies of the plant were procurable.

On 11.9.30 severe symptoms of poisoning were exhibited, namely pronounced apathy, conjunctivae intensely dark pink, head resting on manger, eyes kept half closed, peculiar twitching of the ears, laboured respiration (60 per minute) and yawning.

On 13.9.30 the animal was found pushing its hindquarters against the stable wall, the above symptoms still prevailing. On 15.9.30 constant yawning was noticed and balls of half chewed forage were found in the manger.

There was progressive loss in condition. The animal walked in circles in the stable bumping its head and other prominent parts of the body against the walls. Ultimately the animal presented a pitiful sight. It staggered about in the stable, taking up different abnormal positions and sometimes pushing its head into the corners of the stable. There were ecchymoses on the conjunctivae and a slight rise in the temperature.

On 7.10.30 the stable walls and floor were found covered with blood, wounds on the prominent parts of the body, especially the head. The animal was found standing with spread legs and rocking from side to side. The cornea reflex was absent and the temperature 106.2° F. At 10 a.m. the animal dropped down and a very severe and unsuccessful struggle to rise ensued. The pulse was imperceptible and the respirations extremely accelerated. There were intermittent attacks of asphyxia and the whole body was trembling.

The animal presented such a pitiful sight that it was killed *in extremis*.

Post-mortem Appearances: Anaemia; cachexia; abrasions and wounds over all the prominent parts of the body; subepicardial and subendocardial haemorrhages; marked hyperaemia, slight oedema, and emphysema of the lungs, peri-aortal tissues infiltrated with a colourless gelatinous substance. The liver which was of a pronounced firm consistence, was normal in size, light yellowish-grey in colour and its surface showed protruding nodules, 1 to 2 mm. in diameter. On section these nodules extended into the substance of the liver, were light-grey in colour and had a glassy appearance. Similar nodules were seen throughout the liver substance. The central veins were distended and in certain parts of the liver were surrounded by a dark-reddish zone. There was dilatation of the stomach, which contained very little ingesta, and a slight subacute catarrhal enteritis. Furthermore, it may be pointed out that the liver lesions microscopically resembled a case of the chronic stage of Dunsiekte.

Horse 18969: From 13.2.31 Horse 18969 received daily 60 grams of dry *Senecio* in the pre-flowering stage from Camp C, Westlands, Kokstad.

25.3.31.—Not feeding; pulse 60, strong; respiration laboured and more abdominal; conjunctivae dirty brownish appearance.

26.3.31.—Not feeding; pulse 60, strong; respiration slightly laboured.

27.3.31.—Feeding well; apparently nothing unusual.

28.3.31.—Feeding well; apparently nothing unusual.

1.4.31.—Nothing unusual.

2.4.31.—Nothing unusual.

3.4.31.—Nothing unusual.

4.4.31.—Not feeding well; conjunctivae dark reddish in colour with a yellowish tinge; pulse nothing unusual; respiration markedly laboured; losing in condition.

5.4.31.—Not feeding; conjunctivate dark reddish with yellowish tinge; pulse nothing unusual (48); animal very apathetic; yawning repeatedly; respiration markedly laboured; losing in condition.

6.4.31.—Condition worse than on 5.4.31 and pushing head into corner of the stable; pulse 76, weak.

7.4.31.—Not feeding; animal staggering sideways, falling down and struggling to its feet again; pushing into corner of stable; conjunctivae intensely yellowish; pulse 84, weak; respiration markedly laboured; temperature 101.2° F.; at 4 p.m. animal fell down again and was unable to rise; cornea reflex absent. Died during the night.

Pathological Anatomical Diagnosis

Areas of the integument especially over the eyes devoid of hair with erythema; slight general icterus; cirrhosis of the liver with circulatory changes in the lobuli; marked dilatation of the stomach, degenerative changes of myocardium and kidneys; hyperaemia both lungs.

Microscopically the lesions in the liver were identical with the transition stages of Dunsiekte described by Theiler.

Conclusions.

1. In view of the unconvincing evidence of the field experiments, it is at present not yet possible to conclude that *Senecio* poisoning and Dunsiekte are etiologically one and the same disease.

2. On the other hand, it may be stated that symptomatically and pathologically there is a very close resemblance between *Senecio* poisoning and Dunsiekte, in fact cases were produced by drenching *Senecio* which could not be distinguished from Dunsiekte.

3. In many instances small quantities of *Senecio* in the pre-flowering stage, drenched over short periods were sufficient to set up acute fatal cases of *Senecio* poisoning.

4. From the field experiments it could be concluded that dipping did not check the incidence of the disease, neither did the elimination of *Gastrophilus* larvae prevent Dunsiekte.

5. It would appear that horses which have shown slight symptoms of Dunsiekte may recover without leaving any evidence of liver changes when subsequently destroyed.

6. On the other hand, horses which have never shown symptoms of Dunsiekte on a Dunsiekte farm, may reveal definite liver lesions when destroyed. The appearance of symptoms of Dunsiekte evidently depends on the destruction of a certain amount of liver parenchyma.

Note.—Since the completion of this paper it was found that a large majority of control horses from the Kokstad area (where it was pointed out that *Senecio* is present in large quantities) showed liver lesions when killed although these horses during life never revealed any symptoms. In fact in the experiments completed, probably more than 90 per cent of the control horses on and from the Kokstad farm showed liver lesions. This would indicate that many more horses on a Dunsiekte farm become effected than was thought to be the case up to the present. This observation is undoubtedly very significant and would again seem to indicate the importance of the relation between a Dunsiekte farm and the prevalence of *Senecio* on such a farm. Furthermore, where *Senecio* is less prevalent it is conceivable that liver lesions may be produced in horses without ever being revealed during life.

Acknowledgements.

The writers are much indebted to Mr. Henderson, Government Veterinary Officer, Kokstad, for the way in which he, as far as his work permitted, assisted and supervised the field experiments at Kokstad. The assistance given by Messrs. Appleton, White, Chick and Bachmann, all of the Onderstepoort staff, while in charge of the experiments, is much appreciated and the carefully prepared data by Mr. Bachmann were of great value. The field experiments were made possible by the use of the various camps at Westlands, placed at our disposal by the owner, Mr. Scott-Parkin.

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ADDENDUM.

" Senecio latifolius " and stock- disease.

The Botanical Aspect.

A Contribution by the Division of Plant Industry.

The plant known as *Senecio latifolius* in the sense the species is defined in the " Flora Capensis " (Fl. Cap. III, 377) has always been considered responsible for certain stock-diseases, and while this is correct a more detailed examination of the specimens included under the above species makes it obvious that more than one species is concerned as had already been noted by Phillips (Bot. Surv. Mem. IX, 11, 1927). De Candolle in 1837 (DC. Prodr. VI, 387) described three species of *Senecio*, viz. *Senecio latifolius*, *S. retrorsus* and *S. barbellatus* which Harvey (1864-65) in the Flora Capensis placed together under the name *S. latifolius* DC. though his conception of *S. latifolius* was different and wider than the sense in which De Candolle restricted the species. With further material at the disposal of the Division it is proposed to re-establish De Candolle's three species of *S. latifolius*, *S. retrorsus*, and *S. barbellatus*. As will be seen from the distribution of the species given below, *S. latifolius* DC. is restricted to the coastal area, while *S. retrorsus* DC. is the species which occurs so abundantly in East Griqualand and in the Molteno District and is the plant used in most of the feeding experiments conducted by the Division of Veterinary Services. The plant figured by Walsh (South African Poisonous Plants, p. 28) is *S. retrorsus* DC. and not *S. latifolius* DC. as stated by Walsh, while the plate in Phillips' publication is probably an undescribed species.

In December, 1930, a member of the Division paid a visit to the farm near Kokstad where experiments were being conducted by the Division of Veterinary Services and collected six species of *Senecio*, viz., *S. retrorsus*, *S. othonniflorus*, *S. aduatus*, *S. isatideus*, *S. harveianus*, *S. fraudulentus* and an undescribed species. The

common species in the Kokstad area and which covers large areas of veld is *S. retrorsus*. Two of the species collected at Kokstad, viz., *S. retrorsus* and *S. othonniflorus* also occur in the Molteno District as will be seen from the accompanying maps.

As the question of the species fed in the various experiments conducted by the Division of Veterinary Services is of importance in interpreting the nature of the disease, several allied species of *Senecio* have been examined and their distribution mapped out as far as our available knowledge allows. In the material examined from the various South African herbaria it is found that the name *Senecio latifolius* is used indiscriminately for several distinct species and this has no doubt caused confusion in the feeding tests. The true *S. latifolius* DC. has a restricted coastal distribution from Port St. Johns into Zululand and has probably never been used in the various feeding tests. As will be seen from the distribution maps all the species of *Senecio* under discussion are massed in the eastern part of the Union while only a single species reaches as far west as Knysna. The massing of the species is in Natal and East Griqualand. The Transvaal plant which has so often been named *S. latifolius* is *S. venosus* and has a very restricted distribution, and as far as known does not occur further south than Standerton. The two species, viz., *S. isatideus* and *S. othonniflorus* have the widest and a very similar distribution, occurring in the western Transvaal and sweeping down in a south-easterly direction to Port Elizabeth and Knysna respectively. One species (*S. isatidioides*) has a restricted distribution in the eastern mountain region (see Phillips' Leribe flora under the name *S. macrophyllus* Phillips) and the fact that a supposed case of *Senecio* poisoning has recently been reported from Pietersburg in the Transvaal makes it probable that the above species is concerned and not any of the species occurring in East Griqualand.

Whether or not more than one species of *Senecio* is responsible for similar stock-diseases remains for investigation by the Veterinary Division. It is interesting to note that the late Prof. MacOwan in 1905 received a specimen of *S. retrorsus* from a Government Veterinary Surgeon (no locality cited) as injurious to stock.

In conclusion the Division wishes to thank the Curators of the various South African herbaria for lending the specimens in their collections. The following two plants from the areas concerned proved to be undescribed.

Senecio pachythelis Phill. and Smith n. sp., *Plantaginei* Harv. allied to *S. decurrens* DC from which it differs in the much smaller capitula and the absence of wide stem-wings.

Plant a hemicryptophyte, up to 70 cm. high. *Rootstock* thickened and woody, with numerous spreading-descending tough wiry roots, crowned very frequently by the fibrous remains of old leaf-bases. *Stem* simple, laxly leafy, stoutish and rigid, up to 1.1 cm. in diam. at the base, subflexuous, markedly angled and rib-striate, often somewhat flattened, terminating in a more or less flattopped corymb, glabrous or very sparsely covered with a deciduous toment. *Leaves* mostly radical, coriaceous, prominently veined on both surfaces with close anastomosis of the nerves, finely and more or less evenly serrate along the margins, glabrous or with a loose early deciduous toment;

radical leaves elliptic or broadly lanceolate and long tapering into a more or less flattened herbaceous petiole expanded at the base, acute to obtusely rounded at the apex, up to 24 cm. long (including the petiolar part) and 4 cm. wide; lower cauline leaves similar to the radical; the upper gradually smaller and sessile, eventually reduced to bracts from the axes of which the inflorescence branches arise. *Inflorescence* laxly branched and showy, up to 15 cm. in diam. glabrous in all parts or with a loose deciduous toment on the bractical leaves an dinvolucres; ultimate branchlets up to 6 cm. long, either nude or with 1-3 much-reduced membranous bracts. *Capitula* radiate, about 60-flowered. *Involucre* campanulate; scales 16, 1-seriate, oblong, acute at the apex, thickened on the dorsal face, and with broad scarious margins (the latter often unequal); involucre of about 11 linear acute more or less herbaceous scales surrounding the base of the involucre; receptacle honeycombed, with the margins of the "cells" fimbrilliferous. *Ray-florets* 8; ligules yellow, oblong-elliptic, 3-toothed at the apex, up to 8-nerved owing to splitting of the 4 primary nerves. *Achenes* (immature) oblong, finely striate, glabrous. *Disc-florets* yellow; corolla funnel-shaped in the upper half, with the teeth thickened at the apex, glabrous. *Achenes* oblong, finely striate, glabrous.

Griqualand East.—Mt. Currie Div.: On stony kopje on the farm "Westlands" near Kokstad, December, 1930, *Phillips* 3488 (*Nat. Herb.* No. 8851).

S. INOPHYLLUS Phill. & Smith, sp. nov. (Glaberrimi DC.); allied to *S. barbatus* DC. from which it differs in the very much broader ovate to ovate-oblong leaves.

Plant a hemicrytophyte, up to 60 cm. high, glabrous in all parts except on the rootstock. *Rootstock* thickened and woody, with numerous wiry roots, densely, or sometimes sparsely) woolly-hairy at the crown. *Stems* rigidly erect and flexuous, leafy in the lower part, striate from the decurrent nerves of the leaves, terete, laxly subdichotomously branched at the top to give rise to the corymbose inflorescence. *Leaves* 3-5, distant, broadly ovate to ovate-oblong, rounded at the broadly clasping base and shortly adnate to the stem, appearing perfoliate, gradually narrowed to the acute apex, up to 12 cm. long and 5 cm. wide, dark green in colour, thickly coriaceous, entire-margined (rarely with a few sharp distant-teeth), with prominently raised reticulated nervature on both surfaces; internodes up to 7 cm. *Inflorescence branches* arising from the axes of narrow subulate bracts, flexuous or straight, nude, or with the ultimate branchlets bearing 1-2 very much reduced scales. *Capitula* radiate. Involucre more or less narrowing to the base. *Ray-florets* 3-5; ligules yellow, spreading. *Disc-florets* about 10; corolla subcampanulate in the yellow upper, and cylindrical in the paler lower half. *Achenes* subcompressed, striate, brown, glabrous.

Natal.—Lions River Div.: Lidgetton, Oct. *Mogg* 883! Shale ridges at Caversham, 4,000 ft., Oct., *Mogg* 2457! (Type) Shale ridges at Tweedie, 3,630 ft., Nov.-April, *Mogg* 1185! (Syntype). 1083! 3443! Utrecht Div.: At Volksrust, Jan., *Jenkins in Transv. Mus. Herb.* 10705!

Barberton Dist.: Barberton Mtns. in stony soil in steep hills, June, *Burtt Davy* 320.

The species dealt with in this paper are: —

S. adnatus DC.

S. barbellatus DC.

S. bupleuroides DC.

S. fraudulentus Phill. & Sm. (=*S. Serra* Sond. in Linnaea, XXIII, 68 (1850); and in Harv. and Sond. Fl. Cap. iii, 389 (1864-65) (non Hook).

S. inophyllus Phill. & Sm.

S. isatidoides Phill. & Sm. (=*S. macrophyllus* Phill. in Ann. S.A.Mus. XVI, 154, 1917, non H.B.K.; *S. isatideus* var. *macrophyllus* Thell in Viertelj. Nat. Gesellsch. Zurich. LXVI, 247, 1921).

S. isatideus DC.

S. latifolius DC.

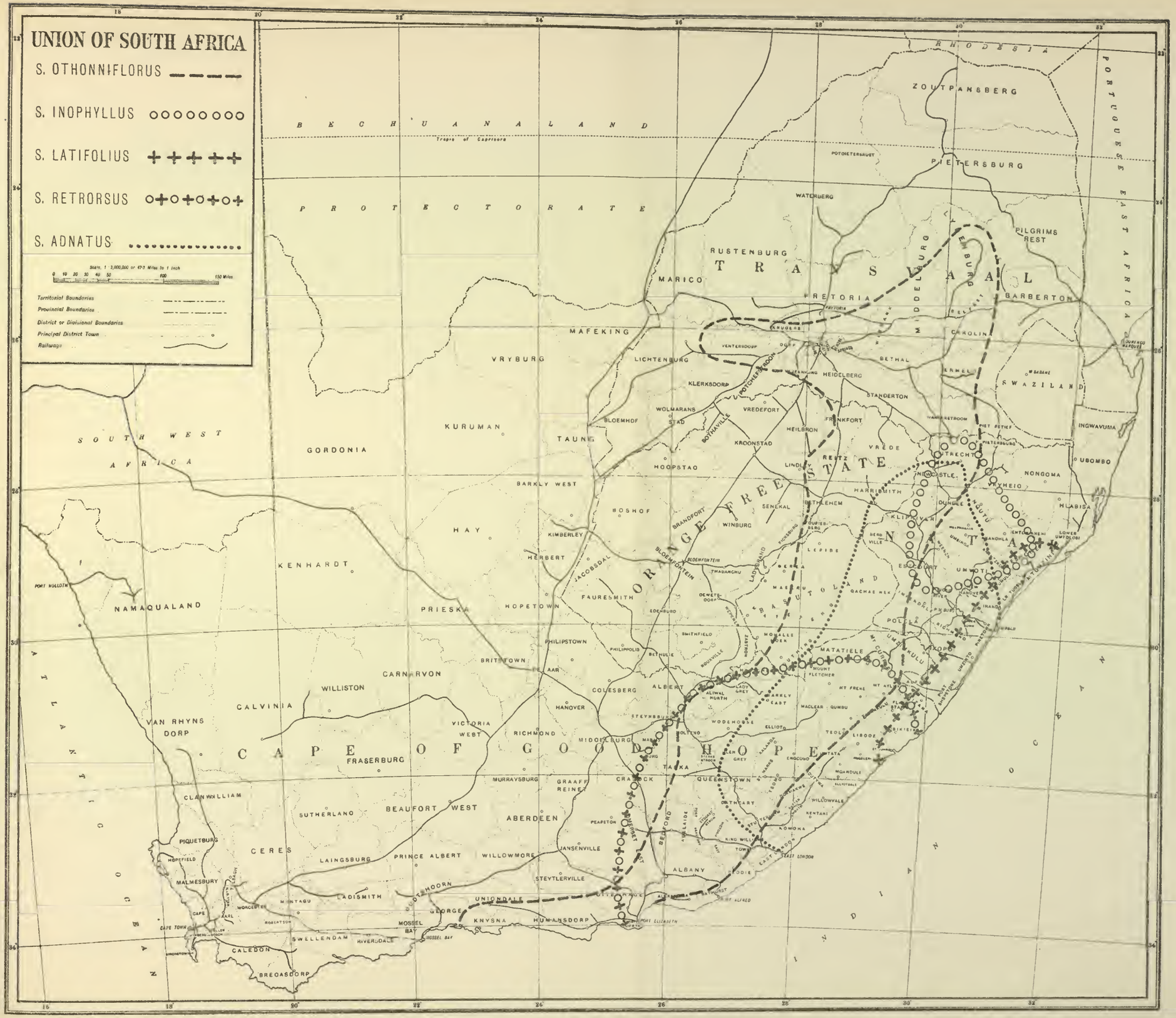
S. othonniflorus DC.

S. retrorsus DC.

S. venosus Harv.

Senecio retrorsus DC.
(Drawn from a specimen in the National Herbarium, No. 8848.)

Senecio pachythelis Phill & Sm.
(Drawn from a specimen in the National Herbarium, No. 8851.)


UNION OF SOUTH AFRICA
S. OTHONNIFLORUS
S. INOPHYLLUS
S. LATIFOLIUS
S. RETRORSUS
S. ADNATUS
Territorial Boundaries
Provincial Boundaries
District or Divisional Boundaries
Principal District Town
Railways
BECHUANALAND
PROTECTORATE
SOUTH WEST AFRICA
RHODESIA
PORTUGUESE EAST AFRICA
ZOUTPANSBERG
PIETERSBURG
WATERBERG
MARICO
RUSTENBURG
TRANSVAAL
PRETORIA
BARBERTON
PILGRIMS REST
MAFEKING
VRYBURG
LICHTENBURG
KLERKSDORP
POTCHEFSTROOM
HEIDELBERG
BETHAL
STANDERTON
SWAZILAND
WOLMARANS STAD
VREDEFORT
FRANKFORT
KURUMAN
TAUNG
BLOEMHOF
KROONSTAD
HEILBRON
VREDE
GORDONIA
HOOPSTAD
LINDLEY
RENTZ
FREE STATE
NEWCASTLE
NONGOMA
INGWAVUMA
USOBOMBO
HLABISA
BARKLY WEST
BOSHOF
SENEKAL
HARRISMITH
BETHLEHEM
KLIPRIVIER
HAY
KIMBERLEY
WINBURG
BRANDFORT
ORANGE
NATAL
KENHARDT
HERBERT
JACOBSDAL
BLOEMFONTEIN
LADYBRAND
LERIBE
ESCOURT
UMTOTI
PORT NOLLOTH
NAMAQUALAND
PRIESKA
HOPETOWN
FAURESMITH
EDENBURG
BASUTOLAND
MATATIELE
PORT SHEPSTONE
PHILIPSTOWN
SMITHFIELD
PHILIPPOLIS
BETHULIE
BRITTOWN
CARNARVON
WILLISTON
CALVINIA
COLESBERG
ALBERT
ALIWAL NORTH
LADY GREY
MT FRERE
MACLEAR
QUMBU
VAN RHYNS DORP
VICTORIA WEST
RICHMOND
HANOVER
MIDDELBURG
STEYNSBURG
WODEHOUSE
ELLIOT
TEOLO
LIBODE
CAPE OF GOOD HOPE
FRASERBURG
TARKA
QUEENSTOWN
CRADOCK
GRAAFF REINET
MURRAYSBURG
CLANWILLIAM
SUTHERLAND
BEAUFORT WEST
ABERDEEN
PEARSTON
WILLOWVALE
KENTANI
KOMGA
PIQUETBURG
CERES
HOPEFIELD
LAINGSBURG
PRINCE ALBERT
WILLOWMORE
JANSENVILLE
KING WILLIAMSTOWN
EAST LONDON
MALMESBURY
STEYTLERVILLE
ALBANY
GRAHAMSTOWN
LADISMITH
UNIONDALE
MONTAGU
WORCESTER
CAPE TOWN
GEORGE
KNYSNA
HUMANSDORP
PORT ELIZABETH
MOSSEL BAY
SWELLENDAM
RIVERSDALE
CALEDON
BREDASDORP
ATLANTIC OCEAN
INDIAN OCEAN

17th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1931.

The Photosensitising Influence of Haematoporphyrin on Sheep and Goats.

By J. I. QUIN, D.V.Sc., Veterinary Research Officer, Onderstepoort.

INTRODUCTION.

In different parts of South Africa, sheep and frequently also goats, are taken ill under peculiar circumstances, and usually only during certain times of the year. The onset of this trouble, which usually manifests itself as a marked swelling of the head and ears to be followed by a general icterus afterwards, is very sudden.

Frequently the cause is known as e.g. in the case of Geeldikkop, which is generally caused by the animals grazing mainly on the common dubbeltjie (*Tribulus terrestris*). The peculiarity, however, is that this plant has to be looked upon as a valuable fodder plant, but which may cause severe losses under certain climatic conditions, and in certain areas only. Thus whereas it is frequently poisonous in the Karroo areas of the Cape Province, it has never been found to produce Geeldikkop in the Transvaal, where it is frequently found growing in large patches. Further, poisonous Tribulus plants when transported from the Cape to the Transvaal and fed to experimental animals even over long periods and in large amounts, have so far never produced a single case of Geeldikkop.

In parts of the Transvaal, Natal and Orange Free State, sheep may, however, take ill with identical swelling of the head, followed by generalised icterus, but on pastures altogether free from Tribulus, and composed practically exclusively of Panicum and Setaria species. Whatever the cause may be, these troubles usually only appear during the rainy season, i.e. from November till March. Cases are, however, sometimes reported in mid-winter. In Merino sheep, swellings are confined exclusively to parts not covered by wool, e.g. the face and ears and sometimes the lower parts of the limbs. In Blackhead Persians, Africanders, and other short-haired breeds, the swelling is confined to the unpigmented and exposed parts of the body, while the pigmented parts, e.g. the blackhead, has never been found to be affected. Thus whatever the actual cause of the disease may be, photosensitisation undoubtedly plays a prominent rôle, effected animals generally seeking shade.

Although numerous experiments have from time to time been conducted in an attempt to reproduce these conditions, only a few, in which Tribulus was fed or allowed to be grazed down in areas where Geeldikkop naturally occurred, yielded definite cases of the disease. Even with Tribulus, positive cases are only produced with difficulty and after repeated attempts. With regard to the other types of the disease, none of these have been produced experimentally.

Due to these difficulties in producing the disease it was decided to attempt other means. The first group to be tried included several dye-stuffs such as methylene blue and eosin solutions, which were injected intravenously into Merino lambs 7-9 months old, and afterwards exposed to strong sunlight.

These experiments were repeated on three different occasions, without any of the animals becoming light sensitive or showing any other effect.

Since Hausmann and other workers had shown that different porphyrins on injection into white mice caused photosensitisation when the animals were exposed in bright light, it was decided to carry out experiments on larger animals, e.g. sheep and goats. For this purpose haematoporphyrin had to be prepared in considerable quantities.

Preparation of Haematoporphyrin.

The method first tried was that of Nencki and Zaleski, in which case haematoporphyrin is prepared from haemin by treatment with hydrobromic acid and glacial acetic acid. Even from fairly large quantities of blood the yield in each case was found to be very small. For this reason it was decided to prepare crude haematoporphyrin by the following method:—

Several litres of freshly drawn, citrated horse blood were allowed to stand over in a series of long glass cylinders for several hours. When sedimentation of the corpuscles was complete, the plasma was carefully siphoned off, and the corpuscles shaken up with large quantities of normal saline. This suspension was then placed in an ordinary cream separator and the red cells separated off at high speed. More saline was added, the corpuscles again shaken up and the performance repeated three or four times, until the cells were fairly well washed. The thick suspension of cells was then poured into a small flask so as to fill it completely, and the flask securely corked. It was then placed in a cool, dark cupboard and allowed to stand for 4 or 5 days. In this way the Oxyhaemoglobin is changed to reduced haemoglobin. To this half decomposed mass of red corpuscles an equal volume of concentrated sulphuric acid is added while continually stirring it. In this way a dark brown syrupy fluid is formed, which, after standing for 6-8 hours, is slowly added to ten volumes of distilled water. The resulting dark reddish violet solution is filtered and the filtrate carefully neutralised with half concentrated solution of NaOH. Just as the neutrality point is reached a dark brownish flocculent precipitate begins to settle out, and only sufficient NaOH now added to cause maximum precipitation. The precipitate is filtered off on filter paper and repeatedly washed with more water until the filtrate no longer reacts with potassium ferricyanide. The washed precipitate after collecting from the filter paper is thinly spread out in flat porcelain vessels and dried in front of an electric fan. The powder which is formed is of a dark brown slightly violet colour. It is only very sparingly soluble in water, although readily so in acids and alkalis, in which it also exhibits some degree of fluorescence. The powder in this form was found to be quite satisfactory for the experiments and hence not purified any further. For injection it was found that the powder readily dissolved in .5 per

cent. sodium bicarbonate solution and then boiled for 3 or 4 minutes afterwards. In this form all the injections were made and as a rule intrajugularly.

EXPERIMENTS WITH CRUDE HAEMATOPORPHYRIN.

A series of preliminary experiments were conducted on guinea-pigs and rabbits. Firstly, adult white guinea pigs were injected subcutaneously under the abdomen with .1 gram Hp. in 5 c.c. .5 per cent. $NaHCO_3$ and adult white rabbits with twice the amount of Hp. and immediately afterwards placed out in bright sunlight. Such animals invariably became restless within 5-10 minutes and soon afterwards started jumping about in their cages. Within 30-60 minutes the lips, nose and eyes started swelling with the skin a bright red colour. After a few hours in the sun, the head and ears were markedly swollen and the eyes closed. In guinea-pigs the point of the nose protruded some distance past the upper lips and giving the animal a "beaked" appearance. At this stage the eyes were usually closed through swelling, respirations were markedly increased and the animals frequently making a squealing sound. If green food, e.g. lucerne, was left in the cage, animals were invariably found buried under it in an attempt to hide themselves from the sun. Unless they were taken out of the sun and placed in the dark, such animals usually died within 6-8 hours. On post-mortem there was a marked oedema of the subcutaneous tissues of the head with congestion of the thoracic and abdominal viscera. If removed from the sun within 1-2 hours, animals usually recovered very soon, although in some cases there is a persistent catarrhal conjunctivitis and also sloughing of the upper layers of the skin of the face. Animals which after injection were placed in the dark, failed to develop any symptoms. Similarly black rabbits and black or brown guinea-pigs, when exposed in the sun after injection, failed to develop the swellings noted in the white animals. After these preliminary experiments, it was decided to ascertain the effect of the same material on sheep and later also on goats.

Experiments on Sheep.

In this series of experiments, a large number of Merino sheep were used over a period of several months during the hottest time of the year, i.e. from October till February. Most of the sheep used were about 9-12 months old and usually in good condition. As a dose of .5 grams crude Haemotoporphyrin dissolved in 20 c.c. of .5 per cent. $NaHCO_3$ injected intrajugularly generally produced quite marked symptoms, this was used as a standard amount for single injections of sheep. Altogether 63 sheep were treated with Hp. at different times and in different experiments. The following were the results:—

EXPERIMENT A.

Five Merino lambs about 7 months old were each injected with 20 c.c. of .5 per cent. solution of ordinary $NaHCO_3$ only, and exposed in sunlight. The object was to note the effect of the weak alkali when injected intrajugularly. As was expected, none of the animals showed any untoward effect.

EXPERIMENT B.

Influence of Sunlight after Hp. Injection.

Five Merino sheep, two lambs 7 months old and three full-mouth sheep, were injected intravenously with .5 gram Hp. on different days, usually between 9 and 10 a.m. and immediately afterwards placed out in the bright sunlight.

Symptoms.—As a rule, within 5-10 minutes after injection the first symptoms were noticeable and in the following order: frequent scratching of the ears with hind legs, shaking of the head and licking of the lips, restlessness, raising of the head with retraction on the spinal column, peculiar backward movement with the head held high, and watering of the eyes with photophobia. The animals by this time were definitely light sensitive, and shade sought, especially for the head, which was frequently kept under the belly of another sheep. There is marked irritation and the head frequently rubbed against the sides of the paddock. Further there is a reddening of the conjunctiva, lips and gums. Respirations are hurried and feeding is stopped. (See Fig. 1.)

Within 1-2 hours there is definite swelling of the ears, later on of the whole face, lips and round about the eyes. This swelling gradually extends to the intermandibular space. The ears now become pendulous and the eyes may be closed. (See Figs. 2 and 3.) Respiration and pulse very hurried and the temperature raised by 3-4° F. The animals may now become very drowsy and listless, although shade is continually being sought for. On cutting into the swollen parts, a clear, pale straw-coloured fluid exudes from the subcutaneous tissues, which are markedly thickened and jelly-like. After 24-36 hours the swelling of the face and ears gravitates to the intermandibular region, which may be very markedly swollen. The skin over the ears, lips and face begins to feel dry and hard, and changed to a dark brown lifeless colour. Frequently there is an opacity of the cornea of both eyes causing either temporary or permanent blindness. Sloughing of the affected skin usually starts after 7-10 days, leaving a pale pink tender surface. (See Figs. 4, 5 and 6.) Frequently the tips of the ears drop off. Even during the acute stages, some animals become very sensitive on their feet, and many of them are too lame to walk. The coronet seems swollen, red and very painful. Frequently an open wound results from which a clear fluid exudes. In rare cases exungulation may result. Animals lose condition very rapidly through constant irritation and inability to feed properly. After a single injection photosensitisation is usually lost after 7 days. Further injections do, however, again bring on acute attacks, which are worse than the first on account of the tenderness of the skin. Only occassionally do such animals die and then usually from asphyxia, following the marked oedema of the head. The post-mortem, except for the head lesions and a generalised congestion of the viscera, is usually negative. Cases showing icterus clinically have never been noticed. The Van den Bergh reaction, which was carried out regularly, did in some cases give a faintly positive indirect reaction, but did not become any stronger even after repeated injections of Hp. From a clinical aspect such animals

closely resemble sheep suffering from Geeldikkop, except that the marked icterus so commonly found with the latter disease is absent from the former.

EXPERIMENT C.

The Influence of Shade after Injections of Haematoporphyrin.

In this experiment young Merino sheep were again injected intravenously with .5 gram Hp. each, and grouped as follows: Two sheep were immediately placed in a dark room after injection and kept there for several days. Two sheep were similarly placed in diffuse daylight inside a stable, and kept there for some days. Seven sheep were placed under a rectangular frame, the top and sides of which were covered by a single layer of hessian. This was kept exposed in strong sunlight and the sides fastened down, so that there was no direct sunlight on the sheep. The animals were fed and watered inside this " box " and not removed from it for several days. All the above animals were kept under close and frequent observation. Not one of them suffered any ill effect. This clearly indicated the effect of even a single layer of hessian in warding off the harmful rays in sunlight.

Some of these animals were removed on the 6th day after injection, and exposed to direct sunlight. In all cases they promptly became photosensitive within 30 minutes and developed mild swellings of the face and ears. The rest of the sheep, which were allowed out after 8 days, failed to become sensitive, indicating that after a single injection of Hp. photosensitisation was lost in about a week.

EXPERIMENT D.

The Influence of Haematoporphyrin on Sheep with Pigmented Skin.

Two black Merino sheep with the skin and wool pigmented all over the body, were injected .5 gram Hp. each, and placed out in bright sunlight and kept exposed for three days. Neither of them suffered the slightest ill effect. They were then again injected with the same amount and again with no effect.

Two half-grown Blackhead Persian sheep were injected .5 gram each and were exposed in sunlight for two weeks. There was no definite photosensitisation visible. After 10 days, however, bare patches started appearing all over the body, especially over the short haired parts, e.g. the inside of the thighs, back of the tail and lower parts of the limbs. At the same time the skin along the back became hard and dry. This also started sloughing after 2 weeks, leaving large open tender surfaces along the back. Never was there any marked swelling noticeable on the body as e.g. seen on the heads of the Merinos. Furthermore, the blackheads of these sheep seemed fully protected since the skin and coat was normal in all respects, there being a sharp line of demarcation between the black and white parts (see Figs. 7 and 8.) Both animals lost in condition somewhat, and one died after some weeks as the result of a worm infestation. Similarly one brown bastard sheep showed no effect after injection and exposure to sunlight. From the above experiment it is clearly evident that skin pigmentation plays an important rôle in preventing photosensitisation after Hp. injections.

EXPERIMENT E.

The Influence of Haematoporphyrin after Artificial Pigmentation of the Skin, and Exposure to Sunlight.

In this case the bare parts of the head and ears of a number of young Merino sheep were carefully pigmented by rubbing in concentrated solutions of various dye-stuffs, and other materials. If after one application the face was not properly pigmented the performance was repeated a second or even a third time. After the skin had dried off, the animal was injected .5 gram Hp. and exposed to strong sunlight, and the effect noted.

The following are the results obtained where a single sheep was covered with a particular dye and then all animals injected afterwards in rapid succession and placed in bright sunlight.

Head Pigmented with:	Observations.
1. Methyl Violet.....	Definitely light sensitive, no marked swelling. Skin sloughing after 7 days. Condition lost.
2. Gentian Violet.....	Fairly sensitive. No marked swelling, skin sloughing after 7 days. Condition lost.
3. Bismarck Brown.....	Slightly light sensitive. No further development, animal normal again.
4. Safranine.....	Definitely sensitive, ears swollen, skin sloughing after week. Animal blind in both eyes. Condition poor —very lame.
5. Haemateine.....	Slightly sensitive, no swelling. Sloughing round lips only.
6. Gum-spirit and Carbon.....	Definitely sensitive, one ear swollen, slight sloughing after 1 week. Feet painful and animal lame.
7. Potassium permanganate.....	Fairly sensitive, ears swollen, sloughing after 1 week. Animal blind—condition poor.
8. Black paint (oily).....	Very sensitive, slight swelling, sloughing slight after 10 days. Feet painful.
9. Tattoo oil (red).....	Easily rubbed off—fairly sensitive, sloughing round nose only. Feet painful.
10. Lithium-carmine.....	Very sensitive—marked swelling next day. Sloughing after 7 days.
11. Chlorophyll (concentrated)...	Green colour easily faded. Markedly sensitive, swelling of head and ears. Marked sloughing. Condition poor.
12. Butter muslin cap tied round the head	Markedly sensitive. Marked swelling. Sloughing after 7 days. Condition poor.
13. Calico cap tied on head.....	Very sensitive, marked swelling and oedema of the whole head. Killed for post-mortem.
14. Normal control placed in sun	Very sensitive, marked swelling of whole head. Sloughing after 1 week. Condition poor.
15. Normal control placed in the shade	No ill effect shown whatever.

From the above experiment it was thus found that in most cases the pigments had exerted some beneficial influence. Thus, although most of the animals were definitely sensitive to sunlight, few of them showed swellings of any significance. Further, except in the case of those covered with chlorophyll, potassium permanganate and carmine, the sloughing which set in after a week, was as a rule fairly mild, and confined to the lips, nose and round about the eyes. The sheep

which was painted with the Bismarck Brown suffered no ill effect, the skin remaining normally plastic throughout (see Fig. 9.) The calico and the butter muslin caps, which were fitted on to two sheep, did not prevent them from becoming markedly sensitive and also swelling severely.

As Bismarck Brown seemed to be the most efficient dye of those used, it was decided to try it on another five sheep. Again the result was similar to that obtained from the first sheep. All five sheep only showed a slight initial irritation in the sun, but suffered no ill effect on the skin afterwards. At the same time a sheep painted with paraphenylenediamine and then injected, only became slightly sensitised and the one ear mildly swollen. Similarly a sheep treated with chrysoidine and injected, showed the same slight effect. Another sheep which was painted with Brilliant Green and injected, became very sensitive and developed a tremendous swelling of the head and ears. It thus seemed that the brown dyes, Bismarck Brown, paraphenylenediamine, and chrysoidine were more effective than the other in preventing the penetration of the harmful rays, while green seemed to be the least effective.

EXPERIMENT F.

The Value of Ordinary Window-pane Glass in Preventing Photosensitisation after Hp. Injection.

In this experiment three pure white Angora goat lambs, 2 months old, were used. Each lamb was injected intravenously with .25 gram Hp.

The first lamb was immediately afterwards exposed in strong sunlight. It became photosensitive within 15 minutes. Soon afterwards the head and ears started swelling. In 24 hours the swelling was at its worst (see Fig. 10.) The eyes were totally closed and the ears about five times the ordinary thickness. The second lamb, immediately after injection, was placed in a wooden box, the lid of which consisted of a sheet of ordinary colourless window pane glass. The box was so arranged that the sun was continually shining through the glass on to the head of the animal. It was then observed carefully for some hours on end. After six hours no definite signs of sensitisation could be detected. It was then placed in a dark stable overnight. The next morning early it was examined. There was a fair swelling of the head and ears, the skin of the ears being about twice the ordinary thickness. The eyes were running slightly (see Fig. 11.) In comparison with the first lamb, the second showed about half the swelling.

The third lamb, which, after injection, was kept in a dark stable all the time, suffered no ill effect at all (see Fig. 12.) It thus seemed that the window-pane glass was capable of cutting out only half of the injurious rays in sunlight.

EXPERIMENT G.

The Influence of Artificially Produced Light Rays after Haematoporphyrin Injection.

1. *Violet Rays from the Quartz Mercury Vapour Lamp.*—Two Merino lambs were injected .5 gram Hp. and immediately afterwards

exposed under a quartz mercury lamp (Hanovia-Artificial Alpine Sun) with the heads kept 24 inches from bulb. The animals were continuously irradiated for hours, without showing the slightest photosensitisation on the head. (Animals were blindfolded to prevent injury to the eyes.) They were then removed and placed in bright sunlight. Within 10 minutes both were strongly photosensitive and vigorously scratching and rubbing their heads. Later on marked swellings developed.

2. *Bright Light from 30,000 Candle Power Carbon Arc Lamp.*— Two young Merino sheep, one of which was injected with Hp. were exposed with the heads 3 feet from the lamp. After 5 minutes the injected lamb started licking its lips, and moving about restlessly. Irradiation was continued for 4 hours with a break of 1 hour in between. At the end, the injected lamb was still showing marked irritation, while the control was standing quietly. The animals were then placed in a dark room overnight, and examined the next morning. The control still appeared perfectly normal, while the other animal showed a small fluctuating swelling in the intermandibular space, apparently as the result of gravitation of the oedema fluid from the face. The rest of the head appeared normal. It thus appeared that, although definite photosensitisation was produced by irradiation from the carbon arc lamp, only slight oedema was formed after some hours.

EXPERIMENT II.

The Effect of Oral Administration of Haematoporphyrin.

One Merino sheep was dosed 5 grams Hp. and placed in the sun. No effect was shown. After 24 hours it was again dosed with the same amount, but again without effect.

SUMMARY AND CONCLUSIONS.

On account of the difficulty experienced in producing cases of Geeldikkop, especially under laboratory conditions, it was decided to ascertain the influence of some photosensitising substances, especially Haematoporphyrin. Since Geeldikkop seems to be a disease definitely associated with photosensitisation, it was thought that perhaps by these means a similar condition could be produced, and which could then be controlled and studied more carefully. Various experiments were conducted with haematoporphyrin and the following are some of the results obtained:—

1. Sheep and goats with unpigmented skins could very easily be rendered photosensitive by exposure to sunlight after intravenous injection of .5 grams Haematoporphyrin.

2. As in Geeldikkop the oedematous swellings and skin lesions were confined to the exposed parts, e.g. to the head and ears and sometimes to the lower parts of the limbs. The symptoms were identical in these two conditions, except that in Geeldikkop a marked generalised icterus is also usually seen, while in Hp. sensitisation this was never found.

3. Animals with patches of pigmented skin suffered no ill effects over such parts, while black sheep showed no effect whatever.

4. If animals are kept out of the sun or very bright diffuse daylight, no effect is produced by haematoporphyrin. Similarly animals placed under a cover of hessian showed no ill effect.

5. Animals could be protected against photosensitisation to some extent by artificial pigmentation of the skin of the exposed parts. Of the different dye-stuffs used, the brown ones, e.g. Bismarck Brown, were found to be the most efficient.

6. In an experiment on young Angora goats it was found that window-pane glass cut off only about half of the sensitising rays in sunlight.

7. Violet rays from the quartz mercury vapour lamp did not produce sensitisation after Haematoporphyrin injection.

8. Irradiation with a powerful carbon arc lamp produced definite sensitisation with slight oedema only.

9. Oral administration of Haematoporphyrin and subsequent exposure to sunlight produced no effect whatever. Further work on this problem is being continued.

I here wish to express my thanks to Mr. M. Carlisle for the assistance rendered in the experiments, and to Mr. T. Meyer for the photographs taken.

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FIG. 1.—Initial stage of photosensitisation with retraction of head and licking of lips, 10 minutes after injection of haematoporphyrin.

FIG. 2.—Acute stage with marked oedematous swelling of face and ears, 4 hours after injection.

FIG. 3.—Acute stage with marked swelling of face and ears, 4 hours after injection.

FIG. 4.—Chronic case recovering with sloughing of the skin over face and ears.

FIG. 5.—Chronic case with dry necrosis of skin of face and ears. Blind in both eyes.

FIG. 6.—Chronic case with hardening and retraction of the lips.

FIG. 7.—Blackhead Persian with loss of skin over unpigmented part of body while black head remains normal.

FIG. 8.—Blackhead Persian with loss of skin over back and hindquarters.

FIG. 9.—Merino with face and ears pigmented with Bismarck Brown. Skin remained normal after injection of haematoporphyrin.

FIG. 10.—Angora lamb showing marked swelling of head and ears, 6 hours after injection of haematoporphyrin and exposure in sunlight.

FIG. 11.—Angora lamb showing fair swelling of head and ears, 24 hours after injection of haematoporphyrin and kept in sun under window-pane glass.

FIG. 12.—Angora goat lamb injected with haematoporphyrin and kept in shade—remained normal.

Section VII.

Sex Physiology.

J. Quinlan and G. S. Maré — The Physiological Changes in the Ovary of the Merino Sheep in South Africa, and their Practical Application in Breeding.

17th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1931.

The Physiological Changes in the Ovary of the Merino Sheep in South Africa, and Their Practical Application in Breeding.

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Sub-Director of Veterinary Services,

and

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The Physiological Changes in the Ovary of the Merino Sheep in South Africa, and Their Practical Application in Breeding.

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INTRODUCTION.

In this paper it is not intended to discuss the physiology of the ovary in the Merino ewe. There are several textbooks and monographs available in which genital physiology is discussed and, although the ewe does not come in for special consideration in these works, no doubt, the general physiological functions attributable to the ovary of other females also apply to the sheep. Within recent years, however, the importance of the ovary as an internal secretory gland has been recognised. The association of special ovarian cells with a definite hormone-secretion has been realised. For instance, the interstitial cells of the ovarian parenchyma are associated with the government of the secondary sexual characteristics. The specialised cells of the Graafian follicle are associated with the morphological and psychological phenomena of oestrus; in other words they produce an oestral hormone.

The primacy of the ovary alone as the regulating factor in the periodicity of the oestrous cycle is questionable in view of recent literature. There is evidence to suggest that the pituitary gland may by periodicity of its own function, be the regulator of the oestrous rhythm. (Novak, 1930.)

The rôle of the corpus luteum as a hormone secreting gland is now fully realised. Its inhibiting influence on follicular development during the inter-oestrous period and during pregnancy, is no longer problematic. The association of the corpus luteum with the preparation of the uterus to receive the fertilized ovum, with the formation of the uterine seal of pregnancy, and with the development of the udder prior to lactation, is an accepted fact. Its association with the pituitary gland in the production of the physiological phenomena of parturition appears to be without doubt. The recent work of Asdell (1928) on the corpus luteum deals fully with every aspect of its important physiological functions. This author also gives a detailed bibliography on the subject. This aspect of ovarian study is not presented here. The authors confine themselves to the morphological changes in the ovary during the sexual cycle, and the psychological phenomena which accompany them, as well as the changes in the ovary during gestation and lactation.

However, owing to the relevancy between the time of ovulation and the vitality of spermatozoa in the genital tract of the ewe to successful breeding, it has been found desirable to deviate somewhat from the pure physiology of the ovary to discuss in some detail the vitality of spermatozoa *in vivo*.

The Merino sheep only will be dealt with, since practical experience in breeding Merinos in South Africa has raised some important problems, which could not be explained by physiological references available in publications of European authors. However, from observations made under field conditions there is no reason to believe that the behaviour of the ovaries of the Merino differs in any way from the other breeds of sheep usually met with in South Africa.

The necessity for carrying out investigations into the physiological changes in the ovary of the Merino ewe in South Africa was prompted by the fact that a large percentage of the stud ewes at the School of Agriculture, Grootfontein, Cape Province, returned to the ram during the mating season. It is necessary to indicate the procedure adopted in serving the stud ewes at the School. During the mating season, which begins in October, the ewes are brought into kraals at 6 a.m. daily. They are tested for oestrus with a number of vasectomised males. Ewes which are in heat are taken to a separate shed and are allowed one service with a chosen stud ram. It will, therefore, be seen that in all cases service took place less than 24 hours after the onset of oestrus. In a large percentage of ewes there is no doubt that service occurred a few hours subsequent to the exhibition of heat.

There was little previous experience with "hand-serving" of ewes in this country, and little literature to guide one as to whether the cyclical ovarian changes of Merino sheep in South Africa correspond with those prevailing in Europe. Küpfer (1928) had published observations on the ovarian cycle of the Merino sheep in South Africa, but practical observations had demonstrated that his conclusions were not entirely satisfactory. He had observed a seasonal period of ovarian activity in Merino sheep, but his observations were not continued over a whole year, and no allowance was made for the variable climate of this country. Observations should refer to an average year, not to an exceptional one. His work was incomplete and, therefore, misleading.

Without experimental investigations, it was assumed that the cyclical changes in the ovary of the sheep in this country were similar to those which prevail in Europe. Marshall and Hammond (1926) had shown that there was no seasonal oestrous cycle in the Merino ewe in the British Isles. Oestrus occurred throughout the year. The duration of oestrus, according to these authors, varies from a few hours to 24 hours, while the interval between one heat period and the next is from fourteen days to three weeks. Küpfer (1928) has also observed that there is no seasonal oestrous period in European sheep. He states that the symptoms of oestrus are apparent for two days. On the third day the symptoms have disappeared. The inter-oestrous period is 21 days. His observations in South Africa showed that the inter-oestrous period was a few days shorter, but the period of oestrus was also two days; shorter or longer oestrus is not mentioned.

Going on the results of these authors' observations, it was, therefore, assumed that a service at any time during the oestrous period between a normal ewe and a normal ram would, as a rule, be followed

by conception. This, however, was not the case. Quite a large percentage of ewes returned to service. Many ewes returned two or three times, and even more often.

It is well known that there are many factors in this country which play an important rôle in successful mating of sheep. Long continued drought, influencing condition, not only causes a low fertility, but in many cases an entire temporary inhibition of ovarian activity. This matter will be referred to later. Obesity is another cause of low fertility, especially in the heavier imported breeds of sheep. Ewes running on poor veld, deficient in phosphates, may also suffer from low fertility.

It has been observed that the percentage fertility of the Merino sheep in South Africa is relatively low as compared with the sheep in Europe (Nicholls, 1926). In observations carried out on 448 Merino sheep, served by the " hand-serving " method, the fertility has been 77.7 per cent. Under less favourable conditions on some of the sheep farms in South Africa fertility is infinitely less. This may be due to a combination of external factors, which causes an upset in the internal organism of the ewe, to be mentioned later, or it may be due to careless breeding in overworking the rams. Overwork or rundown conditions of the ram, influence fertility by causing a lack of vitality in the spermatozoa as suggested by Marshall and Hammond (1926) and Hammond and Asdell (1926).

It may, however, be definitely stated that the sheep on which these observations were carried out were in ideal condition for mating, and that there were no external factors of environment, or food supply, which may account for a general disorder which could be associated with infertility.

In view of the observations recorded by many authors in connection with the viability of spermatozoa in the genitalia, and its relation to the time of ovulation, as a most important factor in conception, it appeared that further investigations were necessary to clear up the cause or causes of sheep repeatedly returning to the ram.

There were two factors which had to be taken into consideration. Firstly, it was possible that the service took place too early during the oestrous period and the spermatozoa had not survived, or at least had become reduced in vitality in the genital tract of the ewe prior to ovulation. Secondly, there was the question of the male being the causal factor in this type of infertility.

It must be explained that infertility was not confined to the females served by one ram. Thirteen stud rams, in good hard condition, were used to serve 448 ewes, an average just less than 35. The range of ewes allowed each ram varied from 16 to 96. Only five rams gave satisfactory results inasmuch as less than 30 per cent. of their ewes returned after first service.

The percentage of unfertilized ewes at the first service must be considered exceedingly high, even in the case of these satisfactory rams.

There were eight rams considered unsatisfactory in that 40, or a higher percentage of ewes returned after the first service. In fact, one ram, No. 13, failed to fertilize any of the 46 ewes mated to him at the first service.

The following table indicates the fertility of thirteen rams mated to 448 ewes using the " hand-serving " method.

TABLE I.

Ram No.	1st Service.				2nd Service.				3rd Service.				4th Service.				All Services.			
	No. Served.	No. Fertilised.	No. not Fertilised.	Percentage Fertilised.	No. Served.	No. Fertilised.	No. not Fertilised.	Percentage Fertilised.	No. Served.	No. Fertilised.	No. not Fertilised.	Percentage Fertilised.	No. Served.	No. Fertilised.	No. not Fertilised.	Percentage Fertilised.	No. of Individuals Served.	No. Fertilised.	No. not Fertilised.	Percentage Fertilised.
1.....	26	22	4	84·6	2	2	0	100·0	—	—	—	—	—	—	—	—	26	24	2	92·3
2.....	23	19	4	82·6	2	1	1	50·0	—	—	—	—	—	—	—	—	23	20	3	87·0
3.....	16	13	3	81·2	—	—	—	—	—	—	—	—	—	—	—	—	16	13	3	81·2
4.....	25	19	6	76·0	6	5	1	83·3	1	1	0	100·0	—	—	—	—	25	25	0	100·0
5.....	96	68	28	70·8	13	6	7	46·2	1	1	0	100·0	—	—	—	—	96	75	21	78·1
6.....	65	39	26	60·0	18	6	12	33·3	5	1	4	20·0	1	0	1	0	65	46	19	70·8
7.....	33	19	14	57·6	10	6	4	60·0	1	0	1	0	—	—	—	—	33	25	8	75·8
8.....	25	14	11	56·0	5	3	2	60·0	—	—	—	—	—	—	—	—	25	17	8	68·0
9.....	55	30	25	54·6	17	9	8	52·9	1	1	0	100·0	—	—	—	—	55	40	15	72·8
10.....	40	13	27	32·5	26	17	9	65·3	1	1	0	100·0	—	—	—	—	40	31	9	77·5
11.....	21	2	19	9·5	6	3	3	50·0	—	—	—	—	—	—	—	—	21	5	16	23·8
12.....	54	4	50	7·4	37	8	29	21·6	4	3	1	75·0	—	—	—	—	54	15	39	27·8
13.....	46	0	46	—	45	4	41	8·8	32	8	24	25·0	4	0	4	0	46	12	34	26·1
TOTAL..	525	262	263	49·92	187	70	117	37·44	46	16	30	34·8	5	0	5	0	525	348	177	—

NOTE.—No account is taken here of the behaviour of the ewe, i.e. a ewe may figure under first service to Ram No. 5, but she may also figure under Ram No. 6 as having been served by him once or even twice, but not fertilized at those services.

The following table indicates the behaviour of the ewes in respect of successive services.

TABLE II.

One Service.			Two Services.				Three Services.				Four Services.				Five Services.			
No. Served.	No. Fertilised.	No. not Fertilised.	Percentage Fertilised.	No. Served.	No. Fertilised.	No. not Fertilised.	Percentage Fertilised.	No. Served.	No. Fertilised.	No. not Fertilised.	Percentage Fertilised.	No. Served.	No. Fertilised.	No. not Fertilised.	Percentage Fertilised.	No. Served.	No. Fertilised.	No. not Fertilised.	Percentage Fertilised.
448	225	223	50·2	187	70	117	37·4	90	44	46	48·9	34	8	26	23·5	6	1	5	16·7

NOTE.—The "not fertilized" ewes at any one service were not all served again at the next service. The total number of ewes in the flock was 475. The "teasing" and mating extended over a period of four months, 1st October–1st February. During that period 27 ewes showed no oestrus; 448 showed oestrus and were served as indicated in the above table, but only 348 were fertilized, i.e. 77·7 per cent. Further, it transpired that there were 13 cases where ewes receives one service and did not lamb; yet they showed no subsequent oestrus, although they were brought to the teasers once a day for twenty days after this service. It is possible that these ewes aborted early during gestation.

The results were so alarming that it became necessary to substitute other rams or transfer the ewes to rams which had given satisfactory results, so that the lambing season should not be continued into the cold winter months.

It must be mentioned that this matter of transfer of ewes to other rams, or substitution of new rams, is to be avoided, if possible. At the commencement of the season the ewes are carefully classed for mating with the most suitable ram. The rams concerned are highly priced stud animals, and it is undesirable that a change of mating should become necessary after the original selection.

In taking a superficial view of the circumstances it would appear that these eight unsatisfactory rams were the direct cause of the infertility in the flocks concerned, but since they got a small percentage of their ewes in lamb at the first and second services, it was possible that there were a combination of factors to explain their failure to impregnate.

The life of the spermatozoa inside the genital tract in relation to the time of ovulation, is most important if sheep are to be " hand-served ", and a ram is to be used on as many ewes as possible. If service took place at the correct time of the oestrous period in relation to ovulation, one service only should be required for conception. Under veld conditions it is customary to mate a ram with 50 ewes in this country; the ram running with the flock on the veld. In this way the ram serves a ewe several times in 36 to 48 hours during the period of oestrus. By the " hand-serving " method, using one service only, an active ram should impregnate 150 to 180 ewes in 3 months and still retain his fertility and his vigour.

It is interesting to compare the results of the ordinary method of mating with those of the " hand-serving " method which are shown in Table I. The percentage lambing in the flocks at Grootfontein, which were under the natural conditions, compare most favourably with that shown in Table I. 1,243 Merino flock ewes were mated to flock rams during November, December, 1929, and January, 1930. 1,196 Lambs were castrated and docked, at the age of 3 weeks, i.e. 96.2 per cent. There were 85 pairs of twins. No records were kept of still-born lambs or of deaths which took place in the flock prior to docking and castrating.

The problem of successful impregnation when mating by " hand-serving " method depends upon the time service should occur during the oestrous period.

Even with a vigorous and good server the 24 hours' test for oestrus and one service has not proved entirely satisfactory. However, there are rams which, although in good health and excellent condition, are weak servers; that is, copulation appears to be incomplete in that the penis is not inserted so deeply into the vagina with the final thrust. It has been with such weak servers that fertility has been particularly low.

Two probabilities existed with such rams. The incomplete service does not allow of the semen being deposited well forward towards the ostium uterinum externum or of the vermiform appendix penetrating the ostium. Several observers, including Marshall and Hammond (1926), maintain that the vermiform appendix enters the ostium during copulation in the ram and is essential to successful impregnation, except in isolated cases.

An experiment has been carried out to ascertain the part played by the vermiform appendix of the ram during copulation, and if it is necessary for impregnation. In South Africa breeders also associate infertility in sheep with absence of the vermiform appendix. The authors could not quite associate themselves with this view, although there is information from a few well-known sheep-breeders, that rams from which the appendix has been removed on account of urethral calculi, a common condition in this country, have afterwards proved good breeders. Not having been in a position to definitely prove or disprove these statements, it was necessary to carry out experimental investigations. The operation was carried out on a Merino ram, T. 417, a proved highly fertile sire. This ram had been previously used in research work by Quinlan, Maré and Ronx (1930), so that potency was known. His record, prior to the operation, may be seen in Table I, Ram No. 2. He was allowed to serve 22 ewes over a period of 35 days following the operation. These services resulted in only 6 ewes being impregnated. In a subsequent test he served 17 ewes over a period of 32 days. These services resulted in 14 ewes being impregnated. In the latter test a control ram was also used to serve 17 ewes over the same period. These services resulted in 16 ewes being impregnated. The control test was done to observe whether mating late in the autumn may not have influenced breeding, in view of the fact that the appendisectomized ram showed a low fertility just after the operation during his first test. It is, therefore, justified to maintain that the vermiform appendix is not an essential factor to successful impregnation in sheep.

It is well known that incomplete coitus may and frequently does result in infertility in other animals. Giles (1919) states that for successful impregnation in women spermatozoa should be deposited at the entrance to or just into the cervical canal, for those that remain in the vagina perish. In humans this appears to be the rule, but exceptions doubtless occur.

Incomplete copulation in sheep, which results in the deposition of semen lower down in the vagina than normal, if not actually into the ostium uterinum, possibly influences the vitality of the spermatozoa, and this in association with an extended time in the genital tract awaiting the ovum may result in their inability to impregnate. In order that these observations should be as complete as possible, an experiment is being conducted to ascertain the distribution and vitality of spermatozoa in the divisions of the genitalia of ewes at intervals after copulation, and also to ascertain how long the motile sperms are capable of fertilizing an ovum made available by ovulation.

It would appear to be not out of place here to discuss shortly some of the literature relevant to this subject under discussion. It has not been possible to find any literature to throw light on this most important question of the viability of spermatozoa in the genital tract of the ewe.

Regarding the duration of viability of spermatozoa in the genitalia of humans the statistics of Giles (1919) are interesting. Haussmann and Hühner (1879), quoted by Giles, maintain the life of spermatozoa in the vagina is not longer than a few hours.

In the cervical canal, Hühner (1913), quoted by Giles, has shown that only in 11.6 per cent. of cases have living spermatozoa been found after 15 to 24 hours; in 20 per cent. after 2 to 5 days; and in 45.9 per cent. after 1 to 12 hours.

The same authors' results of an examination for live spermatozoa in the uterus have shown living sperms in 27 per cent. of cases after 1 to 12 hours; in 16.7 per cent. after 15 to 24 hours; and in 6.3 per cent. after 2 to 7 days.

Regarding the viability of spermatozoa in the Fallopian tubes, Hirschfield, quoted by Bab (1908), found spermatozoa 14 to 16 hours, and Bailey and Miller (1911) found living sperms three and a half weeks after coitus.

In the case of humans, Giles summarizes as follows: "Spermatozoa in the vagina die within one hour after coitus; in the cervical canal they may be found in many cases as long as 2 to 5 days after coitus; in the fundus they are frequently found within 24 hours after coitus, and occasionally after several days. Higher up, that is, in the Fallopian tubes, their normal behaviour is unknown."

Marshall and Hammond (1926), state that the length of time the sperm can live in rabbits varies in different males. It is quite possible that a similar variation may occur in rams. This possibility, coupled with incomplete copulation and delay in the genital tract awaiting the arrival of the ovum, are factors which cannot be lost sight of in connection with fertility in sheep.

The non-occurrence of ovulation until some considerable time has elapsed after service, is recognized as a probable cause of failure to conceive in the mare, but in this animal oestrus may last a full week. Hammond and Asdell (1926) have shown that in rabbits the sperm survived for less than 2 days in the female genital tract. It is quite probable that what is true for the mare and rabbit is also true for the ewe. Marshall and Hammond (1926) believe that with the other farm animals outside the mare this does not likely occur, since the oestrous period is much shorter, as a rule, and the occurrence of ovulation is known to have a more definite time relation to the period of desire.

The same authors recommend that uncertain breeders, especially mares, should be served more than once during oestrus, and it is pointed out that service during the latter part of oestrus is more likely to be fertile than if performed at the commencement.

The experiments on the vitality of spermatozoa in the genitalia of the ewe are not yet completed and, therefore, the results will not be published until a later date. It has been found, however, that the duration of motility of the sperms varies somewhat in individuals, as well as in the different compartments of the genitalia of the ewe. Living sperms have been found in the vagina, and the first few millimetres of the ostium uterinum only 15 minutes after copulation. They have been found in every compartment of the genital tract six hours after copulation. Motile sperms have been seen in the uterine horns 42 hours after copulation only in one case, and they have been seen in the Fallopian tubes after 24 hours. It appears that relatively few of the sperms which are deposited in the vagina ever reach the apices of the horns and the tubes. One may examine a hundred fields before finding a sperm in the tubes. It will therefore be seen that it is possible for some sperms to be present and not be be seen at a microscopic examination. It appears that spermatozoa begin to lose their motility in the genital tract of the ewe before the 24th hour after copulation. Twenty-four hours after service motility has ceased as a rule in the vagina; 50 per cent. only are motile in the cervix; 6 to 10 per cent. only are motile in the uterus; somewhat less than half of those seen in the tubes show motility. It appears probable, therefore, that the fertilizing power of the sperms becomes reduced by a prolonged stay in the genitalia, but it is not possible to say with certainty at what hour after the onset of oestrus service will be followed by the greatest fertility until these experiments are completed.

In South Africa the present observations have shown that oestrus in the ewe may extend three and even up to four full days, with an average duration of about 40 hours. It is, therefore, extremely probable that copulation early during the oestrous period, except with rams in which the sperms are most highly viable, and able to withstand the harmful influences of vaginal, cervical and uterine secretion, will be followed by infertility. Consequently it appears that, to be sucessful, "hand-serving" in ewes, when confined to one service during an oestrous period, should be carried out later than has been the practice here. More successful results will be obtained by a 12 hour test for oestrus and service 18 to 24 hours after its onset. By this practice there is less likelihood of the spermatozoa becoming reduced in vitality by the detrimental influences of the secretions during a prolonged delay in the genital tract. "Hand-serving" by this method would bring the spermatozoa into the uterus a short time before ovulation occurs. It can be assumed that the shorter the spermatozoa are in the genitalia the more vital will they be, since a prolonged stay in contact with genital secretions causes inactivity and ultimate death. It appears likely, therefore, that this method of "hand-serving" will give a highly satisfactory result.

An experiment was carried out to test the most suitable system to follow in practice with "hand-service" of stud ewes. This work was done during the last three months of 1930 with 370 stud ewes and 6 stud rams. The ewes were tested with vasectomised teasers at 6 a.m. daily. All animals showing oestrus were removed from the general flock. Half of the ewes found to be on heat were served at 6 p.m. the same day and the other half at 6 a.m. the next morning.

The following table shows the results of this system of mating.

TABLE III.

Ram No.	Total Services.		Total Unsuccessful Services.		Percentage of Unsuccessful Services.	
	At 6 a.m.	At 6 p.m.	At 6 a.m.	At 6 p.m.	At 6 a.m.	At 6 p.m.
3.....	6	14	3	2	50·0	14·3
5.....	58	72	15	7	25·9	9·7
6.....	17	24	6	3	35·3	12·5
12.....	16	19	7	7	43·8	36·8
14.....	8	17	1	3	12·5	17·6
15.....	8	17	1	0	12·5	0·0
TOTAL.....	113	163	33	22	29·2	13·5

NOTE.—The first four rams above are the same animals referred to in Table I.

The above table shows that rams 3, 5, 6, and 12 had a greater measure of success in impregnating ewes when mated 12 hours after teasing than when mated 24 hours later. The former system must, therefore, be recommended, especially as it was found that 6.4 per cent. of the ewes on heat had lost desire, and refused the rams at the 6 a.m. services, whereas none of the ewes refused service at 6 p.m.

I. OESTRUS AND THE OVARIAN CYCLE.

(A) FACTORS INFLUENCING OESTRUS.

The appearance of oestrus in the Merino sheep in South Africa has been described by Küpfer (1928) as a seasonal occurrence, commencing in January and ending in June or July. It is quite evident, since lambs are born in South Africa in March, April and May, and less frequently in the spring months, September and October, that Küpfer's recorded observations were incomplete. It is the rule for farmers to put the rams with their ewes in October, when the spring rains produce a plentiful supply of good veld. This has the same effect as "flushing" in the British Isles (Marshall and Hammond, 1926). In other words, the ewes are being served during the period when follicular development is favourably influenced by stimulating the ewes with extra feeding. This system has long been recognized as a successful means of attaining the highest percentage of fertility. Within recent years the study of the problems of nutrition in relation to fertility has shown that the fertility vitamin, or Vitamin E, is present in green foods, but not equally in all. For instance, the feeding of sprouted oats has proved successful in overcoming certain types of functional infertility in cattle (Graves and Miller, 1927). Sprouted oats has been recommended for this purpose as being specially rich in Vitamin E.

There is no doubt that Merino sheep in South Africa continue to show oestrus at fairly regular intervals throughout the year, as is the case under European conditions provided the condition is maintained by suitable nourishment. In South Africa the changeable climatic conditions and their relation to the pasturage are factors which govern the cyclical ovarian changes in the ewe. In general it can be stated that there is a continuous cyclical change in the ovary in non-pregnant and non-lactating ewes, when rains are plentiful and pasturage good. Practical experience has taught the farmers that in a bad year the change from succulent to dry pasturage, towards mid-winter, July, may produce a period of ovarian rest. This is continued until the rains again produce a succulent pasturage. The rains begin in September as a rule. In cases of drought, when the spring rains are late, the period of ovarian inactivity may continue until December or even as late as January. It appears probable that it was during a season such as this that Küpfer's observations were made.

The following table indicates the influence of drought on the occurrence of oestrus in the Merino sheep. These records were kept on the same farm during the years 1929 and 1930.

TABLE IV

Month.	Total No. of Flock.		No. Showing Oestrus.		Percentage.	
	1929.	1930.	1929.	1930.	1929.	1930.
October.....	*Varying	370	31	117	—	31·6
November.....	475	370	252	108	53·0	29·2
December.....	475	370	139	15	29·3	4·1
	475	370	422	240	88·8	64·9

* NOTE.—In the season 1929 the large flock was not brought in for teasing until the last week of October, and a fortnight previously only 27 ewes were tested for service to one ram.

In 1930 the teasing commenced on the 6th of October.

The table shows that during 1930 the ewes came into season at a much slower rate than in 1929. In fact during December, 1930, relatively few animals showed oestrus.

This condition is explained from a study of the rainfall during the two years under consideration, as is reflected in the table below. The practice at Grootfontein is to rely on veld-feeding, and for this reason the availability of natural grazing directly influences the condition of the sheep. No supplementary feeding was necessary in the 1929 season, whereas in the 1930 season extra green grazing had to be supplied (mainly lucerne) as well as concentrates. Yet the results were unsatisfactory.

TABLE V.

Rainfall Distribution at Grootfontein, 1929 and 1930, in inches.

Month.	1929.	1930.	Average for 1912–30.
January	1·78	2·08	1·44
February	0·89	0·63	2·08
March	2·28	3·36	2·66
April	0·12	0·24	1·05
May	1·44	0·27	0·47
June	0·41	0·49	0·45
July	1·42	0·02	0·21
August	0·74	0·28	0·30
September	4·01	0·04	0·78
October	1·78	0·47	0·65
November	1·61	0·58	1·23
December	1·97	0·40	1·20
TOTAL	18·45	8·86	12·52

NOTE.—One observes from the above table that in 1929 the rainfall was above normal for three months prior to the commencement of the work and also above normal for every month during which the work was carried out. Under such conditions grazing was exceptionally plentiful and the ewes responded well. During the 1930 season the rainfall was below normal for six months prior to the commencement of the work and also seriously below normal during the three months the ewes were tested. As a result, very little natural grazing was available; in consequence of such adverse conditions the ewes came into season at a much slower rate than under more favourable pastoral conditions.

During such seasons the rams also are influenced. They take little interest and do not search for oestrus in the females. On the other hand, when the pasturage is good throughout the winter, in years when late autumn or early winter rains have occurred, the rams remain active and oestrus in the ewes continues at more or less regular intervals.

It is, therefore, possible for farmers, during an average year with seasonal rains, to produce two crops of lambs. This practice is, however, to be deprecated, as it tends to deteriorate the ewe. It not only influences the vitality of the ewe, but also the wool-production. Further, ewes treated in this way will, after a dual lambing in one year, possibly prove infertile during the following season. Under South African conditions such treatment of Merino ewes cannot be recommended.

It has also been observed that the heavier types of sheep imported into South Africa frequently have a period of ovarian inactivity after arrival. This is no doubt due to the change of environment, that is, climate and pasture. Acclimatisation, as a rule, appears to take about six months. Reference is made here to the Suffolk, Romney-Marsh, Corriedale, Dorset-Horn, and Wensleydale breeds. Not only does the environmental change from Europe to South Africa produce a temporary sexual inactivity, but it frequently follows a sudden change from the humid coastal districts to the dry climate of the Karroo.

Imported sheep during this period of ovarian inactivity frequently put on fat rapidly and become obese. They become physically inactive and do not roam much so that there is a tendency to continue putting on fat. Obesity in sheep, as in other animals, is incompatible with fertility. The ovaries reach a static state; the Graafian follicles fail to develop; the corpus luteum becomes persistent, and it is extremely difficult to overcome the condition. It may be pointed out that the state most conducive to activity of the ovary is a good improving condition before and during the mating season. Malnutrition and obesity are to be rigorously avoided.

Lactation is another factor which influences the psychological exhibition of heat. Heavy milking sheep, which are good mothers, do not show oestrus during the early part of lactation, although ovulation may occur. The drain on the system while nursing the lamb seems to produce silent heat. This passes off with the decrease in the milk-yield or with the weaning of the lamb, which is done in South Africa about the age of four months. It is well known that fertility and ovarian activity are influenced by other factors in nutrition. The importance of a suitably balanced ration with sufficient mineral content has been indicated by du Toit and Bisschop (1929) as essential to high fertility. It appears probable that the infertility in this case is due rather to ovarian inactivity than failure to conceive, although the authors do not specially mention this point. It is likely that ewes will also be influenced in the same manner as cattle if run on veld poor in minerals, such as phosphates.

(b) The Occurrence and Duration of Oestrus.

Regarding the duration of oestrus it has been found that it varies considerably not only in individual ewes, but also in the same ewe at different oestrus periods. Fairly accurate data as regard the duration of this physiological phenomenon have been obtained by testing 60 mature ewes at twelve-hourly intervals. The ewes were tested collectively with five vasectomised teasers at 5.30 a.m. and 5.30 p.m. daily. Ewes showing, or suspected of, oestrus were then tested individually at twelve-hourly intervals until they no longer allowed copulation.

In some of these sheep in which investigations were made regarding the actual time of rupture of the follicle very accurate information was obtained regarding the duration of oestrus. They were tested hourly with the teasers. It was noticed that oestrus came on gradually for some hours before the sheep actually permitted copulation. The teaser was able to pick out sheep coming into heat an hour or two before. The ewe showed a certain increasing desire to copulate, but she did not stand quiet, neither did she run away from the ram. When copulation was once allowed desire was continuous for a time and then again gradually disappeared.

There appears to be an average duration of oestrus of about 40 hours, but it may be as short as 24 hours and as long as 96 hours.

The following tables show in summarized form the results of the experiments on the duration of oestrus and the variation in duration which may be observed at successive oestrus periods.

TABLE VI.

Frequency Distribution of the Duration of Oestrus in Merino Sheep.

No. of Ewes.	*Duration in Hours.*
2	96
2	72
4	60
21	48
24	36
6	24

NOTE.—The above 59 ewes were tested twice daily at twelve-hour periods. The duration was calculated from the hour at which the ewe showed definitely that she would take the ram until the hour she definitely refused service.

TABLE VII.

Showing the Duration of Successive Oestrous Periods in certain of the Individuals referred to in Table II.

Ewe No.	*Duration 1st Oestrus.*	*Duration 2nd Oestrus.*
01	96 hours	60 hours
05	36 ,,	48 ,,
08	36 ,,	36 ,,
010	96 ,,	60 ,,
016	48 ,,	36 ,,
026	36 ,,	48 ,,
032	48 ,,	48 ,,
049	60 ,,	48 ,,
060	48 ,,	36 ,,

NOTE.—The data in the above table would indicate that the duration of oestrus is not constant for all ewes, nor is it constant for any one individual at different oestrous periods.

(c) THE CHANGES IN THE OVARY DURING OESTRUS AND THEIR INFLUENCE ON THE DURATION OF OESTRUS.

In Appendix I, Figs. 18, 19, 20, 21, 1. and 2, there is a series of coloured drawings illustrating the changes of the ovary from the commencement of oestrus until the actual rupture of the follicle. (The commencement of oestrus has been estimated from the time the ewe first allowed copulation.) These drawings illustrate the appearance of the surface of the ovary and also a section through it. An attempt has been made to section the mature follicle and the corpus luteum I in each case. The mature follicle naturally appears somewhat collapsed. In addition, a complete description of each individual figure will be found in Appendix I.

Sheep were killed at frequent intervals from the commencement of oestrus until the rupture of the Graafian follicle.

At the commencement of oestrus there is a well developed Graafian follicle, which may reach a diameter of 1.1 cm. (App. I, Fig. 18, C and D). Its surface capsule is thin-walled and transparent. It is slightly purple in colour. There are usually one or two small blood vessels running across the most prominent part of the capsule. The capsule is tense and ruptures easily on manipulation. Several smaller follicles may be present. The corpus luteum I may be present in the same ovary as the mature follicle, or it may be present in the opposite ovary. In fact both conditions seem to be equally common. There is evidence that the same ovary may ovulate even three times successively, as illustrated in App. I, Fig. 9. This, however, would appear to be uncommon. It is also common for ovulation to take place from both ovaries during one oestrus in the Merino ewe (App. I, Fig. 10). A double ovulation from the same ovary is also seen (App I, Fig. 15). The corpus luteum I remains little changed either in size or colour at the commencement of oestrus (App I, Fig. 18). It may be up to 0.9 cm. in diameter. It is very pale flesh coloured, with a faint yellowish tinge. The greatly reduced corpora lutea of a distinct yellow colour recorded by Küpfer have not been seen at this stage. Cole (1930) states that the corpus luteum of the cow does not show any marked reduction in size until 20-30 days post-oestrum, or, in other words, after another heat period has supervened.

The corpus luteum I changes rapidly in size during the earlier hours of oestrus, and its colour gradually takes on a yellowish tinge. More rapid atrophy still appears to take place towards the time of and immediately subsequent to ovulation (App I, Figs. 1 and 2), accompanying the progressive stage of the more recent corpus luteum.

Marshall and Hammond (1926) state that it is not until the corpus luteum, dating from the last oestrus, has undergone a marked regression, that a new heat period can normally supervene. From our observations, which are illustrated in Appendix I, Fig. 18, a well developed corpus luteum little changed in size or colour from that seen at the mid-term of the interoestrous period, is usually present at the commencement of the new oestrus. As indicated above, the marked regression begins with the commencement of oestrus and becomes more rapid with the bursting of the follicle and the development of a fresh corpus luteum. The corpus luteum II at the time of rupture or just subsequent to it may remain 0.75×0.7 cm. in size as indicated in Appendix I, Fig. 1.

It is not uncommon to find corpora with a small cavity in the centre due to the failure of the luteum cells to grow inwards (App. I, Figs. 13, 17, 20). Such cavities have also been met with in the bovine [Quinlan (1929)]. The evolution of the corpus luteum I, which becomes the corpus luteum II after ovulation, will be described under the heading: "Changes in the ovary during the interoestrous period" in this paper. At the commencement of oestrus the corpus luteum II has become greatly reduced in size; the largest seen has been 0.25×0.2 cm. It has taken on a dark brownish yellow colour. The remnants of a few previous corpora are sometimes represented by brownish pin-point specks on the surface of the ovary (App. I, co. lut. r.).

As the time for ovulation approaches, the capsule of the mature Graafian follicle becomes thinner and more transparent at the most central point of its convexity. It is here that rupture invariably takes place.

The exact time of rupture appears to vary somewhat in individuals. It rarely takes place before the 36th to the 40th hour of oestrus.

It is not possible to record the time of rupture in relation to the length of oestrus, but there appears to be little doubt that it occurs towards the end of the period of oestrus. In these experiments cases have been met with where rupture had not yet occurred at the 40th hour.

The duration of oestrus appears to depend on the time of rupture of the follicle. This delay in rupture appears to explain the prolonged oestrus occasionally observed; that is, oestrus extending to three or even four days. This long period must be considered above the average for Merino sheep. Some six hours after rupture of the follicle the desire to copulate gradually passes off.

The importance of this fact in the " hand-serving " of sheep has already been referred to in the introduction to this paper.

In the sheep which were destroyed for these investigations, it is interesting to note that both ovaries were closely invested by the ovarian pocket. The whole ovary appears to be loosely covered by this anatomical structure, and the fimbriated exteremity of the Fallopian tube closely applied to the ovary near the point of rupture of the mature Graafian follicle.

(d) Periodicity of Oestrus and the Duration of the Inter-Oestrous Period.

In considering the investigations into the periodicity of oestrus, it is necessary to explain how these observations were carried out. In the large flock of 448 stud sheep on which the work was begun, the ewes were tested for oestrus daily at 6 a.m. Sheep showing heat were then served with the stud rams. One service only was given at an oestrous period. It has been mentioned in the introduction to this paper that sheep returned to some of the rams once, twice or even more frequently. It is on these sheep which returned that the first observations were carried out, so that the time given represents the periodicity of the occurrence of oestrus rather than the inter-oestrous period.

The following table illustrates the periodicity of oestrus.

Table VIII.

Showing the Frequency Distribution of the Length of the Oestrous Cycle in Merino Sheep.

No. of Ewes.	*Length in Days.*
1	6
1	8
1	12
1	13
1	14
4	15
31	16
82	17

TABLE VIII—(*continued*).

No. of Ewes.	*Length in Days.*
64	18
23	19
4	20
2	21
1	22
1	26
1	27
1	34
5	35
1	36
4	37
1	38
1	41
1	44
1	51
1	68
TOTAL 234	

NOTE.—The figures above are for 234 different individuals, i.e. successive oestrous cycles are not included.

In the 60 sheep on which the observations were made regarding the duration of oestrus, the actual duration of the inter-oestrous period —the time between the disappearance of one oestrus and the commencement of the next—as well as the actual duration of the oestrous period are given. A sheep was considered as showing oestrus when the ram was allowed to copulate and oestrus was considered ended when copulation was no longer allowed. The gradual coming on and gradual going off is not considered, so that the inter-oestrous period is the time between the end of one oestrous period and the commencement of another.

The following table illustrates the duration of the inter-oestrous period in the 60 sheep where very accurate data are available.

TABLE IX.

Inter-oestrous Period in Merino Sheep.

No. of Ewes.	*Duration in Days.*
1	12½
1	13½
4	14
8	14½
11	15
10	15½
9	16
2	16½
1	17

NOTE.—Observations were begun on 60 ewes, but 13 were killed in connection with our observations on the changes in the ovary. The 12½ and 13½-day ewes above both had oestrous cycles of 16½ days, while oestrous lasted for 96 and 72 hours respectively.

In the case of the two 16½-day ewes, oestrus lasted 36 and 48 hours, while the cycles were 18 and 18½ days respectively.

The 17-day ewe above had a cycle of 18½ days and oestrus lasted for 1½ days.

Table X illustrates the varying periodicity of oestrus which may be observed in individual sheep at successive oestrous periods.

TABLE X.

Successive Oestrous Cycles in Days of Twenty-four Merino Flock Ewes.

Ewes No.	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th
0· 1	18	17	17	18	17	18	16	17	16	—	—	—	—
0· 3	16	17	18	17	18	16	17	15	18	17	—	—	—
0· 5	17	18	18	17	17	18	17	17	17	18	18	*	—
0·10	16	16	17	16	18	15	16	17	15	16	17	17	—
0·11	17	17	—	34	17	17	17	16	17	17	—	34	17
0·14	18	19	18	17	18	17	18	16	19	19	*	—	—
0·15	17	17	17	17	17	17	16	17	17	—	35	—	—
0·24	17	17	17	—	33	—	34	16	16	17	—	34	—
0·25	16	17	18	17	18	16	16	17	17	18	17	17	—
0·26	18	18	17	18	17	18	18	17	—	36	17	*	—
0·34	16	17	16	16	16	16	—	33	15	17	17	*	—
0·35	17	17	17	17	16	17	17	16	17	17	17	*	—
0·39	17	17	17	17	16	18	—	32	17	16	17	17	17
0·41	17	17	18	18	17	17	17	17	18	18	—	—	—
0·43	17	18	17	18	17	17	17	23	17	18	*	—	—
0·49	17	17	18	17	17	18	16	16	16	18	17	17	17
0·50	17	17	17	16	17	17	16	18	17	17	17	17	—
0·52	16	—	—	—	65	16	—	33	16	17	—	32	17
0·53	16	17	17	17	17	19	—	30	17	17	—	—	53
0·55	16	17	16	—	34	16	16	—	35	17	17	17	—
0·56	17	17	16	17	17	18	—	31	17	17	—	—	—
0·60	17	18	18	18	17	18	18	16	17	18	18	*	—
0·61	—	—	—	68	18	—	32	16	18	17	—	—	—
0·62	17	18	17	18	17	17	17	18	17	18	—	36	—

* Slaughtered for investigational purposes.
— Silent oestrus?

NOTE.—The testing for oestrus extended over the period 3rd February to 24th September, 1930. The animals grazed on veld and received no extra feeding. The data in the above table would indicate that:—

1. In no case was the cycle absolutely constant.
2. Remarkable uniformity exists in the length of the oestrous cycle, except in the case of ewe 0.43.
3. Regular occurrence of oestrus was not maintained to the same extent towards the close of the experiment; this was probably due to the poor condition of the veld.

(E) THE CHANGES IN THE OVARY DURING THE INTER-OESTROUS PERIOD.

In Appendix I the daily changes in the ovary during the inter-oestrous period are illustrated by means of coloured drawings (Figs. 3 to 17). The description of individual figures is given in some detail. The ovarian changes during oestrus in the Merino ewe have been illustrated in Appendix I. Figs. 18, 19, 20, 21, 1, and 2.

In describing the actual changes which take place in the ovary during the inter-oestrous period, it is proposed to commence shortly after the rupture of the follicle—such a condition is illustrated in Appendix I, Fig. 1, *a*, *b*, *c*, *d*,—and end at the onset of oestrus, which is illustrated in Appendix I, Fig. 18. The changes in the intervening period, namely during oestrus, are illustrated in Appendix I, as referred to previously.

At the time or rupture of the mature Graafian follicle the point of rupture is indicated by an opening with somewhat rugged borders. The border is stained with coagulated blood (App. I, Fig. 1 *c*), a small blood clot rapidly closes the opening. Haemorrhage does not occur at once into the follicular cavity (App. I, Fig. 1 *d*). The walls of the ruptured follicle collapse, but the cavity still retains some clear fluid. Aschoff (1924) has indicated that haemorrhage does not take place in the human ovary after rupture.

A few hours subsequent to rupture the wall of the Graafian follicle gradually increases in thickness, and grows inwards to fill the cavity of the follicle (App. I, Fig. 2 *d*). This filling in of the cavity has taken place as a rule 24 hours after the cessation of oestrus, or roughly 30 hours after ovulation. The lutein tissue rapidly becomes vascularised by the ingrowth of blood vessels from the theca. At the time of rupture the follicular wall is white in colour with some haemorrhagic points, but it rapidly becomes pinkish-red coloured as the corpus fills the cavity (App. I, Fig. 3 *d*).

The now developing corpus luteum I projects above the surface of the ovary as a rosette-shaped dark reddish prominence. This projection may reach a height of 0.3 cm. and a diameter of 0.6 cm. (App. I, Fig. 4 *c*). The ovary at first appears plump, but retains its normal oval shape. It increases gradually in size and in weight until about the mid-term of the inter-oestrous period when it may reach a weight of 2.25 grams. The shape of the ovary also changes from an oval to a cone, rounded, or prism-shaped body.

The corpus luteum I appears to reach its maximum size towards the mid-term of the inter-oestrous period; that is, from the 6th to the 8th day (App. I, Figs. 9, 10, 11). It is nearly always sphere-shaped or slightly oval. Occasionally there is a prism-shaped tendency. It reaches a diameter on section of 1.1×1.0 cm. The rosette-shaped prominence gradually begins to disappear and the corpus sinks into the ovarian tissue, where it remains little reduced in size until the beginning of the next oestrous period. At this time it may measure 0.9×0.9 cm. on section. The prominence of the corpus luteum disappears altogether towards the later part of the inter-oestrous period or is represented only by a small yellowish white, fat-like piece of tissue (App. I, Fig. 17 *c*). At the beginning the corpus luteum I is reddish-pink coloured. It gradually shows a slight change, becoming paler, but remains pale reddish pink coloured throughout the inter-oestrous period. Just prior to the next oestrus a slight tinge of yellow develops. The changes of the corpus luteum during the oestrous period have already been described. Two corpora lutea I may be found in this same ovary in the Merino ewe. The corpus luteum I and II may be found in the same ovary, indicating successive ovulations from one ovary. This is a common occurrence. It is, however, more common to find the corpus luteum I in one ovary and corpus luteum II in the other.

At the time of ovulation there may be some fairly large Graafian follicles present in the same ovary or in the opposite ovary (App. I, Fig. 1 *b*). It is the rule for one follicle to increase rapidly in size during the first few days after ovulation, so that it is possible to say which will be the next mature follicle. The developing follicle at this

stage may reach a diameter of 0.75 to 0.86 cm. Two follicles may show this rapid development. They may both be in one ovary or one may be situated in each ovary. The maturing follicle gradually increases in size and its capsule becomes more transparent. As the liquor folliculi increases it becomes more tense. The appearance of the maturing follicle from the commencement of oestrus has already been described and illustrated in Appendix I, Figs. 18 *c*, *d*, to 21 *a*, *b*.

During these investigations the ovaries of different individuals have been carefully measured and weighed at twenty-four-hour, or more frequent, intervals during the ovarian cycle. As a rule, it may be taken that the ovaries are lightest just subsequent to ovulation and heaviest when the corpus luteum I reaches its maximum development, that is, from the sixth day of the inter-oestrous period. The heaviest ovaries are those which contain the largest corpora lutea. The largest corpus luteum measured was 1.2×1.0 cm. on section on the 13th day of the inter-oestrous period. The ovary weighed 1.98 grams. The heaviest ovary weighed 2.25 grams. on the 10th day of the inter-oestrous period. The corpus luteum I which it contained measured 1.1×1.0 cm. The lightest ovaries weighed 0.67 and 0.63 grams respectively at or just subsequent to ovulation.

The measurements of the ovaries were taken as follows: (1) length from pole to pole, (2) depth from the attached to the free border, (3) width from the median to the lateral face.

The range of these measurements and the range in weights of the right and left ovaries appear in the following table.

TABLE XI.

* Measurement in Cm.						* Weight in Grams.	
Left.			Right.			Left.	Right.
1.	2.	3.	1.	2.	3.		
1·35–	1·10–	0·80–	1·25–	1·00–	0·80	0·63	0·67
2·20	1·85	1·35	2·00	1·70	1·35	2·09	2·25

* There is little use in estimating the average measurements and weights since both depend entirely upon the state of development of the corpus luteum I.

II. PREGNANCY.

(A) THE CHANGES IN THE OVARY DURING PREGNANCY AND EARLY LACTATION.

The changes in the ovary in the Merino ewe during pregnancy have also been observed during these investigations. The corpus luteum vera, or that of pregnancy, has been studied at intervals from the date of copulation until the time of parturition. The appearance of the corpus luteum vera is illustrated in Appendix II, Figs. 1 to 7.

It will be readily seen, if the corpora lutea I illustrated in the coloured drawings in Appendix I, Figs. 1 to 21, be compared with the corpus luteum vera which is illustrated in Appendix II, Figs. 1 to 7, what a great difference exists between the corpus luteum vera and the corpus luteum menstruations. The former at the first month of pregnancy (App. II, Fig. 2) is almost always slightly oval or sphere-shaped. It is somewhat prominent; the prominence is rounded, not abrupt or sharply defined. It appears dark mauve or purple in colour through the capsule. On section it has quite a distinct appearance, being darker red in colour than the corpus luteum spuria. It has a distinct colour of freshly cut muscle. The mid-section is circular or very slightly oval in outline. It may reach a measurement of 1.4×1.0 cm.

As pregnancy advances to the end of the second month (App. II, Fig. 3) the corpus luteum vera becomes more embedded, so that the surface of the ovary becomes more even. On section there is a slight variation in size and it becomes darker red in colour. The measurements may vary up to 1.2×1.2 cm. [The illustration selected (App. II, Fig. 3) to represent the corpus luteum vera at the end of the second month shows a corpus in each ovary. There was a foetus in each uterine horn].

At the end of the third month of pregnancy (App. II, Fig. 4), the corpus luteum may show slight variation in size up to 1.2×1.15 cm. in diameter. It is dark red in colour. [The corpus illustrated (App. II, Fig. 4 *d*) shows a small cavity in its centre]. It has now become well embedded so that the surface of the ovary appears smooth.

Towards the end of the fourth month (App. II, Fig. 5) the corpus luteum begins to decrease in size. There is, however, slight variation. It remains dark red in colour. It has become well embedded, so that its presence is not always easily detectable through the surface of the ovary.

At the end of the fifth month or approaching the time of parturition (App. II, Fig. 6), the size of the corpus luteum vera has decreased slightly. There is also a commencing change in colour. At this stage it is brownish red.

At the time of parturition usually between the 146th to the 152nd day, there is marked change in colour in the corpus luteum. It has taken on a brownish yellow appearance (App. II, Fig. 7). Further, it has decreased slightly in size. The corpus luteum illustrated in Appendix II, Fig. 7, was circular in outline and measured 0.8 cm. in diameter.

Immediately after parturition there is a rapid change in the size of the corpus luteum vera. Its colour remains brownish-yellow. One or more Graafian follicles rapidly develop in preparation for the first post-parturition ovulation. (App. II, Fig. 9.)

The time of ovulation after parturition varies somewhat. It appears to occur between the 10th and the 15th day. A case of ovulation has been seen on the 12th day postpartum (App. II, Fig. 10), while a follicle a short time after rupture has been seen on the 14th day (App. II, Fig. 11). At the time of rupture of the follicle, 10 to 15 days after parturition, the remnant of the corpus luteum vera

(App. II, Figs. 10 and 11) is greatly reduced in size, and it has taken on a brownish colour. At this time it differs from the corpus luteum II of the oestrous period (App. I, Fig. 1), in that the latter is of a pale yellow colour. Subsequent to the formation of the corpus luteum I the history of the regressive corpus luteum vera does not differ from the corpus luteum II of the inter-oestrous period.

It appears that, although ovulation may occur within 10 to 15 days following normal parturition, it is not accompanied as a rule by the psychological exhibition of heat when the ewe suckles her lamb. However, should the lamb be still-born ovulation accompanied by normal oestrus may occur as early as 17 days after parturition. Parkes (1929) says there is no cyclical ovarian change during lactation in the sheep and, therefore, supports these observations in so far as the exhibition of heat is concerned.

Further observations are being carried out on sheep immediately following parturition, and it is hoped to be able to publish more extensive data as to whether post-parturition ovulation is sometimes accompanied by the symptoms of oestrus even in ewes suckling their lambs.

The measurements and weights of the ovaries have been carefully taken during the period of gestation. It may be taken, as a rule, that the ovary containing the corpus luteum vera remains relatively the same size and weight during the first four months of pregnancy. The size of the ovary depends on the presence of the corpus luteum vera. Towards parturition with regression of the corpus the size and weight of the ovary decrease. The ovary which has not ovulated at the oestrus at which conception occurred remains more or less static during pregnancy. The Graafian follicles remain small and unaltered. This ovary is smaller and lighter than the ovary at any time during the inter-oestrous period.

The range of these measurements and the range in weights of the right and left ovaries during gestation appear in the following table.

TABLE XII.

* Measurement in Cm.						* Weight in Grams.	
Left.			Right.			Left.	Right.
1.	2.	3.	1.	2.	3.		
1·40–1·95	0·70–1·65	0·55–1·10	1·50–2·10	1·00–1·50	0·55–1·20	1·28–	1·61–

(B) DURATION OF PREGNANCY.

While these observations were being carried out an opportunity was afforded to study the duration of pregnancy in the Merino ewe in this country. No previous reference could be found to the duration of gestation under South African conditions. Reference from other countries are so numerous that they cannot all be given here. It

appears sufficient to indicate that there appears to be a considerable variation not only in different breeds of sheep, but also in sheep of the same breed. Tessier, quoted by Brown (1902), observed the gestation period in 912 ewes. The following are the results of his observations:—

"140 lambed between 146 and 150 days, mean period 148 days; 676 lambed between 150 and 154 days, mean period 152 days; 96 lambed between 154 and 161 days, mean period 157 days."

Von Nathusius, also quoted by Brown, gives an average period of 150.3 days in the Merino ewe.

Stoss (1928) does not make any differentiation between the different breeds of sheep. He gives the average period of gestation as 150 days.

Craig (1930) goes into some detail regarding the period of gestation in sheep. He quotes several authors as follows:—

"In 429 observations, Magne gives the shortest period as 143 days, and the longest 156 days, the average being 149 days. Colin gives the average as 151 to 152 days; Dietericks gives the shortest period as 146 days, the longest 157; average 151 days; and Baumeister and Rueff state the shortest period in the sheep and goat as 135 days, the longest 160 days; average 144 days. In Merinos the period of gestation averages 150.3 days, and in Southdown 144.2 days."

Marshall and Hammond (1926) state: "The duration of pregnancy is usually reckoned as twenty-one weeks, but the early maturing breeds, such as the Southdown, have a slightly shorter period than the late maturing ones, such as the Merino; the duration of pregnancy is nearly always between 140 and 160 days, but the variation is, as a rule, much less."

The following table shows the gestation period of 338 ewes, which were used in our experiments.

TABLE XIII.

Frequency Distribution of Gestation in Merino Sheep.

Number.	*Duration in Days.*
1	142
1	143
3	144
6	145
20	146
34	147
53	148
66	149
62	150
51	151
27	152
9	153
1	154
3	155
1	156
GRAND TOTAL .. 338	

NOTE.—From the above data it would appear that gestation in Merino sheep varies to the extent of at least 15 days. The average duration may be considered to be from 146-152 days with the majority at 149 days. It must be pointed out that the above were all normal births.

Since data are available concerning the sex of lambs born after different periods of gestation, the following table has been compiled.

TABLE XIV.

Sex in Merino Sheep in Relation to Duration in Pregnancy.

Duration of Pregnancy.	*No. of Males.*	*No. of Females.*
142 days	1	0
143 ,,	1	0
144 ,,	1	0
145 ,,	2	2
146 ,,	4	14
147 ,,	13	19
148 ,,	29	17
149 ,,	30	28
150 ,,	20	28
151 ,,	22	20
152 ,,	19	6
153 ,,	5	3
154 ,,	1	0
155 ,,	1	0
TOTAL	149	138

NOTE.—The figures in the above table do not bear out convincingly the belief that ewe lambs are born at shorter pregnancy duration that ram lambs, as both sexes were born at nearly all periods. However, if 142-147 days are taken as short periods, then the majority of lambs born for those periods are females, i.e. 35 females to 22 males. Similarly, if 152-155 days are considered as long periods, then the majority are males, i.e. 26 males to 10 females.

Before concluding this paper the authors would like to express their gratitude to Mr. E. Parish, Principal of the School of Agriculture, Middelburg, C.P., for having given them facilities to carry out these researches; to Messrs. P. D. Rose, E. N. S. Warren, N. G. Wessels, V. T. Jelliman, and W. A. Stahl, Sheep and Wool Officers, for their co-operation in helping to collect data; and to Mr. Walker, artist at this Institution, for the excellent illustrations he has reproduced from the fresh ovaries supplied to him.

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(*a*) LIST OF ABBREVIATIONS.

Co. lut. I = Corpus luteum of the latest intervulation period

Co. lut. I_1 = One of the two corpora lutea of the latest interovulation period.

Co. lut. II = Corpus luteum of the second latest interovulation period.

Co. lut. r. = Corpus luteum of third latest or of previous interovulation periods.

Co. lut. v. = Corpus luteum of pregnancy.

Co. lut. Vr. = Regressive corpus luteum of pregnancy.

Fo. Gr. = Graafian follicle.

Fo. Gr. dil. = Enlarged Graafian follicle.

Fo. Gr. mt. = Mature Graafian follicle.

Fo. Gr. rpt. = Ruptured Graafian follicle.

(*b*) APPENDIX I.

The Changes in the Ovary during the Oestrous Cycle.

This appendix gives details of ewes slaughtered for observation during the investigations. The series of changes which occur in the ovary during the sexual cycle are illustrated by coloured drawings. A full description of each figure is also given. The coloured drawings represent the changes in the ovary at intervals from the rupture of the mature Graafian follicle seen in Fig. 1 to the 26-hour of oestrus (Fig. 21) prior to rupture of the mature follicle at the next oestrous period.

The measurements and weights of the ovaries of individual sheep are also detailed. The measurements are taken, (1) from pole to pole, (2) from the attached to the free border, (3) from the median to the lateral face. It is in this order that the measurements appear in the description of the figures in the appendix. This system of measurement applies to the ovaries in both the appendices.

FIG. 1, *a*, *b*, *c*, *d*.

Merino ewe, No. 20; date, 5.3.30, 9 a.m.
Duration of last interoestrous period, 14½ days.
Duration of last oestrus, 60 hours.
Duration of present oestrus, 41 hours.
During the present oestrus the ewe had been served once by the teaser at the commencement, and again at the 38th hour.

Left Ovary, Figs. *a* and *b*—

Measurements, 1.6×1.15×1.15 cm. Weight, 0.95 gm.

Fig. *a*.—The corpus luteum II is very prominent. It is pale yellow in colour. It protrudes above the ovary as a nipple-shaped prominence 0.45 cm. in diameter and 0.3 cm. in height. There are several small Graafian follicles present, the largest being 0.5 cm. in diameter.

Fig. *b*.—The corpus luteum II is pale yellow in colour. It is oval in outline and measures 0.75×0.7 cm. The maturing follicle described in Fig. *a* has also been sectioned. It appears somewhat collapsed.

Right Ovary, Figs. *c* and *d*—

Measurements, 1.25×1.1×0.8 cm. Weight, 0.67 gm.

Fig. *c*.—The Graafian follicle has just ruptured. The point of rupture is indicated by an opening in the capsule with jagged borders. The borders are blood-stained. The point of rupture is level with the surface of the surrounding ovarian capsule, not raised above it. There are several small Graafian follicles present.

Fig. *d*.—Section shows the cavity of the recently ruptured follicle. Its wall appears "furred", with several small reddish spots, the remainder being white or yellowish white. It appears as if there had been several small haemorrhagic points on the wall of the ruptured follicle.

FIG. 2, *a*, *b*, *c*, *d*.

Merino ewe, No. 30; date, 27.2.30, 9.30 a.m.
Duration of last interoestrous period, 14½ days.
Duration of last oestrus, 48 hours.
Duration of present oestrus, 40 hours.
During the present oestrus the ewe had been served once by the teaser at the commencement, once at the 24th hour, and once again at the 36th hour.

Left Ovary, Figs. *a* and *b*—

Measurements, 1.5×1.25×1.0 cm. Weight, 0.85 gm.

Fig. *a*.—The surface of the ovary shows several developing follicles of varying size.

Fig. *b*.—Section shows some small developing follicles bulging on the surface.

Right Ovary, Figs. *c* and *d*—

Measurements, 1.75×1.15×1.1 cm. Weight, 1.35 gm.

Fig. *c*.—Ovulation has already occurred. It is impossible to say when actual rupture took place, but it was a very short time previous to slaughter, as the blood around the point of rupture was not an organized clot. The point of rupture is raised somewhat above the surface of the surrounding follicular capsule. The actual ruptured area is circular and 0.2 mm. in diameter. Its border is irregular and saturated with blood, so that it forms a reddish circle. The follicular fluid (liquor folliculi) does not appear to have entirely escaped, as the cavity has not entirely collapsed. The capsule around the rupture is yet transparent, so that the extent of the follicle is still recognizable. The corpus luteum of the previous interovulation period is present, just visible on the surface of the ovary as a whitish yellow elevation. Some Graafian follicles, varying in size, are also visible through the surface.

Fig. *d*.—Section shows the corpus luteum II (that of the completed inter-ovulation period) and the recently ruptured follicle. The follicular wall has become thickened, 0.25 cm. It is almost white in colour and soft, but does not break down like a clot when raised with a mounting needle. There is a small amount of colourless fluid present in the cavity, but no clotted blood. The cavity measures 0.3 cm. in diameter. The only blood coagulum present is attached to the borders of the actual rupture in the follicular wall.

The corpus luteum of the previous interovulation period has undergone rapid change since the commencement of oestrus. It has become much smaller, 0.55 cm., and has changed in colour to very pale yellow.

FIG. 3, *a*, *b*, *c*, *d*.

Merino ewe, No. 48; date, 8.3.30, 10.30 a.m.
Period since termination of oestrus, 12 hours.
(The ewe still showed symptoms of oestrus at 6 p.m., 7.3.30, but was off at 10 a.m., 8.3.30.)

Duration of oestrus, 48 hours.

Left Ovary, Figs. *a* and *b*—

Measurements, 1.8×1.25×1.2 cm. Weight, 1.63 gm.

Fig. *a*.—The corpus luteum II is very prominent. It protrudes above the surface of the ovary for a distance of 0.25 cm. It measures 0.5 cm. in diameter at the highest part of the prominence. It appears through the capsule as a pale yellow body. Several small Graafian follicles are present.

Fig. *b*.—The corpus luteum II measures 0.7×0.6 cm. It is slightly oval in outline and pale yellow in colour.

Right Ovary, Figs. *c* and *d*—

Measurements, 1.8×1.3×0.9 cm. Weight, 1.28 gm.

Fig. *c*.—The follicle has ruptured a short time, probably 12 to 18 hours. The point of rupture is covered with a blood-clot measuring 2 mm. in diameter and 1.5 mm. in height. Four remnants of previous corpora lutea appear as brownish specks on the surface. There are several small Graafian follicles of varying size present.

Fig. *d*.—The corpus luteum I measures 0.5×0.5 cm. It is very pale pink in colour. The cavity is entirely filled. Just at the point of rupture of the capsule it protrudes above the surface of the ovary as a reddish prominence. The red staining does not extend deeply into the corpus, but is entirely superficial. On close examination the surface of the blood-stained area present an uneven appearance. A few small Graafian follicles appear towards the surface.

FIG. 4, *a*, *b*, *c*, *d*.

Merino ewe, No. 2; date, 21.2.30, 9 a.m.
Period since termination of oestrus, 24 hours.
Duration of oestrus, 48 hours.

Left Ovary, Figs. *a* and *b*—

Measurements, 1.4×1.15×0.925 cm. Weight, 0.75 gm.

Fig. *a*.—The surface shows some well-developed Graafian follicles, the largest of which reaches a diameter of 0.85 cm. There are 3 brownish red pin-point areas, the remnants of previous corpora lutea.

Fig. *b*.—Shows a section through a fairly large Graafian follicle. There is an embedded corpus luteum II, measuring 0.55×0.5 cm., which was not visible through the surface. It is pale yellow in colour and presents a slightly oval outline.

Right Ovary, Figs. *c* and *d*—

Measurements, 1.55×1.4×0.9 cm. Weight, 0.98 gm.

Fig. *c*.—The corpus luteum I projects 0.25 cm. above the surface of the ovary. It presents a rosette-shaped appearance, 0.6 cm. in diameter, with slightly irregular and somewhat ragged border. It is deep red in colour. The corpus luteum r. appears as a pale yellowish nipple-like prominence. There are several small developing Graafian follicles, varying in size up to 3 mm., visible through the surface.

Fig. *d*.—On section the corpus luteum I measures 0.6×0.6 cm. It is more or less circular in outline and flesh-coloured. The corpus luteum r. measures 0.45×0.3 cm. It is somewhat oval in shape and pale-yellow in colour. One fairly large Graafian follicle has been sectioned.

FIG. 5, *a*, *b*, *c*, *d*.

Merino ewe, No. 7; date, 20.2.30, 9 a.m.
Period since termination of oestrus, 1½ day.
Duration of oestrus, 48 hours.

Left Ovary, Figs. *a* and *b*—

Measurements, 1.65×1.35×1.1 cm. Weight, 0.95 gm.

Fig. *a*.—The corpus luteum projects 2 mm. above the surface of the surrounding ovary as a flattened circular prominence. It is deep red in colour. The corpus luteum II appears as a pale yellow nipple-shaped body. There is a well-developed Graafian follicle also present.

Fig. *b*.—On section the corpus luteum I measures 0.65×0.65 cm. It is more or less circular in outline and flesh-coloured. The cut surface presents a slightly grooved appearance at its centre. The corpus luteum II measures 0.5×0.55 cm. It is almost circular in outline and is pale yellow in colour.

Right Ovary, Figs. *c* and *d*—

Measurements, 1.5×1.3×0.8 cm. Weight, 0.85 gm.

Fig. *c*.—The surface shows three well-developed Graafian follicles, the largest of which reaches a diameter of 0.7 cm. There are several smaller follicles. There are four brownish-red pin-point areas, the remains of corpora lutea of previous interovulation periods.

Fig. *d*.—Shows a few Graafian follicles on section. The corpus luteum r. appears as a small wedge of tissue, brownish in colour.

FIG. 6, *a*, *b*, *c*, *d*.

Merino ewe, No. 12; date, 21.2.30, 2 p.m.
Period since termination of oestrus, 3 days.
Duration of oestrus, 36 hours.

Left Ovary, Figs. *a* and *b*—

Measurements, 1.35×1.2×0.8 cm. Weight, 0.63 gm.

Fig. *a*.—The surface presents several small developing Graafian follicles. The corpus luteum II is scarcely visible as a small nipple-shaped prominence very pale yellow in colour.

Fig. *b*.—On section the corpus luteum II measures 0.5×0.5 cm. It is circular in outline and very pale yellow in colour. There are some small Graafian follicles evident on the surface of section.

Right Ovary, Figs. *c* and *d*—

Measurements, 1.4×1.55×1 cm. Weight, 1.02 gm.

Fig. *c*.—The presence of the corpus luteum I changes the shape of the ovary, which presents a triangular appearance. The apex is represented by the protruding portion of the corpus luteum. The corpus luteum I protrudes above the surface of the ovary for a distance of 0.6 cm. There is no well-defined line of demarcation, the corpus luteum running smoothly into the surface of the ovary. A few small blood-vessels can be seen at its junction with the ovary. The projecting portion of the corpus luteum is dark red in colour. It is somewhat cone-shaped. Towards its apex it is 0.45 cm. in diameter. There are two well-developed Graafian follicles present: 1, 0.75; 2, 0.6 cm. in diameter.

Fig. *d*.—On section the corpus luteum I has a somewhat conical outline. It measures 0.85×0.8 cm. It is pale flesh-coloured. In its centre there is a small elongated cavity filled with clear fluid. A large Graafian follicle has been sectioned.

FIG. 7, *a*, *b*, *c*, *d*.

Merino ewe, No. 38; date, 22.2.30, 9 a.m.
Period since termination of oestrus, 4 days.
Duration of oestrus, 36 hours.

Left Ovary, Figs. *a* and *b*—

Measurements, 1.75×1.5×1.2 cm. Weight, 1.4 gm.

Fig. *a*.—The corpus luteum I protrudes above the surface of the ovary for a distance of 0.4 cm. It presents a rosette-shaped appearance. It is 0.625 cm. in diameter. The colour is dark red. There is a fairly well-marked line of demarcation between it and the surface of the ovary. The corpus luteum II appears as a small nipple-shaped prominence, pale yellow in colour. Some small Graafian follicles are also apparent through the surface.

Fig. *b*.—On section the corpus luteum I is slightly oval in outline. It measures 1.0×0.9 cm. It is pale flesh-coloured. The corpus luteum II measures 0.4×0.3 cm. The periphery is very pale yellow in colour. Towards its centre there is a brownish colour which increases in depth from the periphery towards the centre.

Right Ovary, Figs. *c* and *d*—

Measurements, 1.925×1.0×0.85 cm. Weight, 0.95 gm.

Fig. *c*.—The surface of the ovary presents an irregular outline due to the presence of several well-developed Graafian follicles. Its anterior pole shows a Graafian follicle, 0.75 cm. in diameter, which is marked off from the remainder of the ovary by a distinct neck.

Fig. *d*.—Shows a section through the largest Graafian follicle. Several developing follicles appear on the surface. The corpus luteum r. has also been sectioned. It is 2 mm. in diameter. The periphery is white, while its centre is brownish, the depth of colour increasing towards the centre.

FIG. 8, *a*, *b*, *c*, *d*.

Merino ewe, No. 21; date, 24.2.30, 9 a.m.

Period since termination of oestrus, 5 days.

Duration of oestrus, 36 hours.

Left Ovary, Figs. *a* and *b*—

Measurements, 1.5×1.4×1.1 cm. Weight, 1.33 gm.

Fig. *a*.—The surface of the ovary shows several developing Graafian follicles, the largest of which reaches a diameter of 0.7 cm. Two brownish-red pin-point specks, the remnants of corpora lutea of previous interovulation periods, can be seen.

Fig. *b*.—Section shows several developing Graafian follicles. The wall of the large follicle mentioned above bulges on the surface of section. It has just been missed with the knife.

Right Ovary, Figs. *c* and *d*—

Measurements, 1.475×1.7×1.3 cm. Weight, 1.53 gm.

Fig. *c*.—The presence of corpus luteum I gives the ovary a distinctly cone-shaped appearance, the apex of the cone being represented by the protruding corpus luteum. There is no line of demarcation between the protruding portion of the corpus luteum and the ovary. Towards the junction several small blood-vessels are apparent. The corpus luteum projects above the surrounding ovary for a distance of 0.4 cm. Its diameter at the most prominent part is 0.55 cm. Its rosette-shaped protrusion is flesh-coloured. The corpus luteum II can be recognized on the surface of the ovary as a small pale yellow prominence, which scarcely rises above the surrounding tissue. Several small Graafian follicles in the developing stage are present.

Fig. *d*.—On section the corpus luteum I appears as an oval pale flesh-coloured body, measuring 0.9×0.8 cm. The corpus luteum II measures 0.35×0.4 cm. It shows a white fibrous-looking periphery and a very pale brownish centre, the depth of colour increasing towards the centre.

FIG. 9, *a*, *b*, *c*, *d*.

Merino ewe, No. 8; date, 1,3.30, 9 a.m.

Period since termination of oestrus, 6 days.

Duration of oestrus, 36 hours.

Left Ovary, Figs. *a* and *b*—

Measurements, 1.5×1.85×1.35 cm. Weight 1.8 gm.

Fig. *a*.—The presence of corpus luteum I gives the ovary a somewhat cone-shaped appearance, the apex of the cone being represented by the protruding corpus luteum. There is a slight constriction at the base of the protrusion of the corpus. Several small tortuous blood-vessels appear in the ovarian capsule covering the embedded portion of the corpus luteum. The protruding portion of the corpus measures 0.6 cm. in diameter and 0.4 cm. in height. It is pale flesh-coloured. There is a large maturing Graafian follicle present, which measures 0.7 cm. in diameter. Some small follicles can also be seen. The corpus luteum II is evident as a small nipple-shaped pale yellow prominence. This ovary has ovulated at two successive oestral periods, and it would appear as if a third successive ovulation would take place from the fact that the largest maturing follicle is present.

Fig. *b*.—Shows the corpus luteum I, which measures 1.15×0.9 cm. It presents an oval outline. It is pale flesh-coloured. The corpus luteum II is also seen. It is circular in outline with a white periphery and a brownish centre. It measures 0.45×0.45 cm. An enlarged Graafian follicle has also been sectioned.

Right Ovary, Figs. *c* and *d*—

Measurements, 1.5×1.2×1.0 cm. Weight, 1.1 gm.

Fig. *c*.—The surface of the ovary shows several small developing Graafian follicles. Three very small brownish red points representing the remnants of previous corpora lutea are present.

Fig. *d*.—Shows a section through the ovary. Some small Graafian follicles bulge on the surface.

FIG 10. *a*, *b*, *c*, *d*.

Merino ewe, No. 23; date, 24.2.30, 2 p.m.

Period since termination of oestrus, 7 days.

Duration of oestrus, 36 hours.

Left Ovary, Figs. *a* and *b*—

Measurements, 2.2×1.25×1.1 cm. Weight, 1.75 gm.

Fig. *a*.—The surface of the ovary shows several developing Graafian follicles. There are two brownish red pin-point areas, remnants of previous corpora lutea. Towards the posterior pole of the ovary there is a small flesh-coloured somewhat conical elevation. This is apparently the corpus luteum, which appears to be almost entirely embedded. Several small tortuous blood-vessels are present in this area.

Fig. *b*.—Section shows corpus luteum I, which measures 1.0×0.8 cm. It is oval in shape and light flesh-coloured. There are several developing follicles present.

Right Ovary, Figs. *c* and *d*—

Measurements, 2.0×1.2×1.1 cm. Weight 1.35 gm.

Fig. *c*.—The surface of this ovary does not differ from the left. There is a somewhat similar elevation, apparently corpus luteum I, which is embedded in the ovary. Both ovaries appear to have ovulated at the recent oestrous period.

Fig. *c*.—Presents an entirely similar appearance to Fig. *b*. The corpus luteum I measures 1.0×1.0 cm. It is pale flesh-coloured. There is a small rounded cavity in its centre which contains fluid. Several developing follicles can be seen.

FIG. 11. *a*, *b*, *c*, *d*.

Merino ewe, No. 22; date, 4.3.30, 9 a.m.

Period since termination of oestrus, 8 days.

Duration of oestrus, 36 hours.

Left Ovary, Figs. *a* and *b*—

Measurements, 2.0×1.5×1.3 cm. Weight, 1.95 gm.

Fig. *a*.—The corpus luteum I projects above the surface of the ovary as a rosette-shaped prominence. It is flesh-coloured. The rosette-shaped prominence is 0.75 cm. in diameter and it stands above the surrounding ovarian capsule for a distance of 0.4 cm. There is a developing Graafian follicle 0.55 cm. in diameter. The corpus luteum II is also present. It is just visible on the surface as a pale yellowish brown speck. Some small Graafian follicles are also present.

Fig. *b*.—The corpus luteum I is oval in outline, measuring 1.0×0.9 cm. It is pale flesh-coloured. The corpus luteum II measures 0.3×0.25 cm. It shows a white fibrous-looking periphery and a brownish medulla. The large developing follicle described in Fig. *a* has not been sectioned. It bulges on the surface of section.

Right Ovary, Figs. *c* and *d*—

Measurements, 1.3×1.1×0.8 cm. Weight, 0.8 gm.

Fig. *c*.—Several small Graafian follicles are seen on the surface. Towards the posterior pole of the ovary there is a prominent follicle, which measures 0.5 cm. in diameter. Some remnants of previous corpora lutea are present on the surface.

Fig. *d*.—Some small Graafian follicles are present, as well as the larger follicle described in Fig. *c*, which has not been sectioned.

FIG. 12, *a*, *b*, *c*, *d*.

Merino ewe, No. 32; date, 4.3.30, 2 p.m.

Period since termination of oestrus, 9 days.

Duration of oestrus, 48 hours.

Left Ovary, Figs. *a* and *b*—

Measurements, 1.4×1.7×1.2 cm. Weight, 1.47 gm.

Fig. *a*.—The corpus luteum I is present. It changes the ovary from an oval to a somewhat cone-shaped body, the apex of the cone being represented by the slightly protruding portion of the corpus luteum. Through the capsule the corpus luteum presents a purple or dark mauve colour. There are some small Graafian follicles present. The corpus luteum II is just visible through the surface as a yellowish brown speck.

Fig. *b*.—Shows a section through the corpus luteum which is pale flesh-coloured. It measures 0.9 cm. in diameter. The corpus luteum II is also sectioned. It shows a white periphery and a brownish centre. Some small Graafian follicles are also present.

Right Ovary, Figs. *c* and *d*—

Measurements, 1.6×1.1×0.95 cm. Weight, 0.98 gm.

Fig. *c*.—Shows several Graafian follicles, varying in size. The maturing Graafian follicle is present in this ovary. It is 0.65 cm. in diameter.

Fig. *d*.—Section shows some small Graafian follicles bulging on the surface. The maturing follicle is also sectioned. The brownish remnants of a corpus luteum II are also recognizable.

FIG. 13, *a*, *b*, *c*, *d*.

Merino ewe, No. 16; date 3.3.30, 2 p.m.

Period since termination of oestrus, 10 days.

Duration of oestrus, 36 hours.

Left Ovary, Figs. *a* and *b*—

Measurements, 1.5×1.2×1.0 cm. Weight, 1.05 gm.

Fig. *a*.—The surface of the ovary shows some small Graafian follicles of varying size.

Fig. *b*.—Shows a section of the ovary with some small Graafian follicles.

Right Ovary, Figs. *c* and *d*—

Measurements, 1.8×1.55×1.35 cm. Weight, 2.25 gm.

Fig. *c*.—The corpus luteum I is present. It gives the ovary a somewhat rounded shape. There is a slight projection of the corpus luteum above the surface of the ovary. There is no marked line of demarcation; the corpus runs smoothly into the surrounding ovarian capsule. There are several Graafian follicles present, the largest of which reaches about 0.75 cm. in diameter.

Fig. *d*.—Shows a section through the corpus luteum I. It is more or less circular in outline, measuring 1.0×1.1 cm. It is pale flesh-coloured. There is a small irregular cavity which is filled with fluid. The cavity is placed a little to one side of the centre. A small follicle is also present. A corpus luteum II was also present in this ovary, but since it was not apparent through the surface it was missed when sectioning. It measured 0.3×0.2 cm.

FIG. 14, *a*, *b*, *c*, *d*.

Merino ewe, No. 17; date, 3.3.30, 9 a.m.

Period since termination of oestrus, 11 days.

Duration of oestrus, 36 hours.

Left Ovary, Figs. *a* and *b*—

Measurements, 1.4×1.1×0.8 cm. Weight, 0.7 gm.

Fig. *a*.—Several Graafian follicles of varying size are apparent. The corpus luteum II is present. It protrudes somewhat above the surface of the ovary as a nipple-shaped whitish yellow prominence. Some brownish specks indicate the remnants of previous corpora lutea.

Fig. *b*.—Shows a section through corpus luteum II, which measures 0.25×0.3 cm. It is oval in outline. Its periphery is white, while the centre is yellowish brown, the depth of colour increasing towards the centre. A corpus luteum r. is also present. It is very dark brown in colour. Some small follicles are also present.

Right Ovary, Figs. *c* and *d*—

Measurements, 1.6×1.5×1.3 cm. Weight, 1.5 gm.

Fig. *c*.—The presence of corpus luteum I changed the shape of the ovary. It has a somewhat rounded appearance. The corpus protrudes somewhat above the surface of the surrounding ovary, but there is no well-defined line of demarcation. The ovarian capsule covering the corpus shows a few small blood-vessels. There are a few remnants of previous corpora lutea indicated as brownish specks. Several small Graafian follicles are also present.

Fig. *d*.—Shows a section through corpus luteum I. It measures 0.95×0.95 cm. It is pale flesh-coloured. There are remnants of previous corpora indicated by yellowish and brownish specks. Some small Graafian follicles are also present.

FIG. 15, *a*, *b*, *c*, *d*.

Merino ewe, No. 59; date, 6.3.30, 2 p.m.

Period since termination of oestrus, 12 days.

Duration of oestrus, 36 hours.

Left Ovary, Figs. *a* and *b*—

Measurements, 2.15×1.2×1.35 cm. Weight, 2.09 gm.

Fig. *a*.—There are two corpora lutea I present. They are close together and project somewhat above the surface of the surrounding ovary, as nipple-shaped prominences with a slight depression in the centre. Through the capsule they appear mauve in colour. There are some Graafian follicles also present. These vary in size, the largest being 0.5 cm. in diameter.

Fig. *b*.—The corpus luteum I (1) measures 0.8×0.8 cm. The corpus luteum I (2) measures 1.0×0.7 cm. Both are pale flesh-coloured. Some small follicles are also seen on the surface of section.

Right Ovary, Figs. *c* and *d*—

Measurements, 1.4×1.2×0.9 cm. Weight, 0.8 gm.

Fig. *c*.—There are two maturing follicles separated only by a thin septum, (1) 0.65 cm., (2) 0.6 cm. in diameter. The corpus luteum II is just visible on the surface as a pale yellowish speck. Some small Graafian follicles are also present.

Fig. *d*.—The corpus luteum II measures 0.5×0.45 cm. It shows a pale periphery and a yellowish centre. One of the Graafian follicles described in figure *c* has been sectioned; the other bulges on the surface of section.

FIG. 16, *a*, *b*, *c*, *d*.

Merino ewe, No. 42; date, 5.3.30, 2 p.m.

Period since termination of oestrus, 13 days.

Duration of oestrus, 36 hours.

Left Ovary, Figs. *a* and *b*—

Measurements, 2.0×1.35×1.2 cm. Weight, 1.98 gm.

Fig. *a*.—The corpus luteum I is easily recognizable as a rounded somewhat flat prominence. The protruding portion is 0.8 cm. in diameter and 0.35 cm. above the surface of the surrounding ovarian capsule. It is dark mauve-coloured. There are three developing Graafian follicles: (1) 0.725 cm., (2) 0.7 cm., (3) 0.5 cm. It would appear as if the two large follicles would rupture at the next oestrus if the sheep had been allowed to live. The remnants of three previous corpora lutea are evident. Some small follicles are also present.

Fig. *b*.—The corpus luteum I is somewhat oval in outline, measuring 1.0×0.9 cm. in diameter. It is pale flesh-coloured. The largest follicle described in figure *a* has also been sectioned.

Right Ovary, Figs. *c* and *d*—

Measurements, 1.5×1.3×0.9 cm. Weight, 0.81 gm.

Fig. *c*.—The corpus luteum II is present as a small pale yellowish rounded prominence, 0.375 cm. in diameter. There is a distinct neck at its attachment to the ovary. Several small Graafian follicles are present, but none of them are so large as the three described in Fig. *a*.

Fig. *d*.—Shows a section through corpus luteum II. It has been pushed towards the surface of the ovary by a Graafian follicle which is developing underneath. The corpus is pale yellow in the centre with a white or whitish yellow periphery.

FIG. 17, *a*, *b*, *c*, *d*.

Merino ewe, No. 37; date, 6.3.30, 9 a.m.

Period since termination of oestrus, 14 days.

Duration of oestrus, 48 hours.

Left Ovary, Figs. *a* and *b*—

Measurements, 1.4×1.3×1.1 cm. Weight, 0.95 gm.

Fig. *a*.—There are four large follicles present, measuring (1) 0.8 cm., (2) 0.65 cm., (3) 0.5 cm., (4) 0.4 cm. There are traces of two remnants of previous corpora lutea as brownish specks.

Fig. *b*.—Shows a section through the largest follicle described in figure *a*. A smaller follicle also bulges on the surface of section.

Right Ovary, Figs. *c* and *d*—

Measurements, 1.4×1.15×1.05 cm. Weight, 1.05 gm.

Fig. *c*.—This ovary contains the corpus luteum I. It is embedded and covered by the ovarian capsule, through which it presents a somewhat mauve colour. A few tortuous blood-vessels are present in the capsule. At one point there is a slight nipple-shaped prominence of a pale yellow colour. Close to this prominence is a cyst-like structure, 0.5 cm. in diameter, apparently a developing Graafian follicle. Some small Graafian follicles can also be seen through the surface of the ovary.

Fig. *d*.—Shows a section through the corpus luteum II, which measures 1.0×0.9 cm. It is somewhat oval in outline and pale flesh-coloured, with a slightly yellowish tinge. In its centre is a somewhat rectangular cavity, filled with fluid, which measures 0.25×0.2 cm. The corpus luteum II is also present. It appears as a yellowish oval structure, which is not visible on the surface.

FIG. 18, *a*, *b*, *c*, *d*.

Merino ewe, No. 44; date, 25.2.30, 9.30 a.m.

Period since termination of oestrus, 15 days.

Duration of last oestrus, 48 hours.

This sheep was killed 15 minutes after oestrus appeared. She was served twice by the teaser just prior to slaughter.

Left Ovary, Figs. *a* and *b*—

Measurements, 1.7×1.3×1.0 cm. Weight, 1.27 gm.

Fig. *a*.—The corpus luteum I is visible through the surface. It is level with the surrounding ovarian surface, except for a very small prominence, which is pale yellowish in colour. Some small tortuous blood-vessels are visible in the ovarian capsule covering the corpus. There are several small Graafian follicles present.

Fig. *b*.—Shows the corpus luteum I, which is circular in outline and very pale flesh-coloured. There is possibly a pale tinge of yellow. It measures 0.9×0.9 cm.

Right Ovary, Figs. *c* and *d*—

Measurements, 1.9×1.3×1.1 cm. Weight 1.25 gm.

Fig. *c*.—There is a large Graafian follicle, which measures 1.1 cm. in diameter at the posterior pole of the ovary. The capsule is thin and transparent. A small blood-vessel can be seen running across the most convex part of the capsule. Some small developing follicles are present.

Fig. *d*.—Shows a section through the large Graafian follicle described in Fig. *c*. Some smaller follicles also bulge on the surface of section.

FIG. 19, *a*, *b*, *c*, *d*.

Merino ewe, No. 31; date, 25.2.30, 4 15 p.m.

Duration of last interoestrous period, 14 days.

Duration of last oestrus, 60 hours.

Duration of present oestrus, 6 hours.

The ewe was served by the teaser during oestrus.

Left Ovary, Figs. *a* and *b*—

Measurements, 1.7×1.5×1.3 cm. Weight, 1.4 gm.

Fig. *a*.—Ovulation has not yet occurred. The ripe Graafian follicle measures 0.875 cm. in diameter. Its capsule is thin and transparent. A small blood-vessel runs across the follicular capsule. The corpus luteum I is also apparent. It protrudes very slightly above the surface of the surrounding ovary. The protrusion is very pale yellowish. Some small follicles are also present.

Fig. *b*.—Section shows the corpus luteum I, which measures 0.75×0.75 cm. It is very pale flesh-coloured, with a faint yellowish tinge. The large Graafian follicle has also been cut through.

Right Ovary, Figs. *c* and *b*—

Measurements, 1.6×1.3×1 cm. Weight, 0.95 gm.

Fig. *c*.—Several Graafian follicles of varying size may be seen through the surface of the ovary. The corpus luteum II is visible as a brownish speck.

Fig. *d*.—Section shows the corpus luteum II, which measures 0.25×0.2 cm. It is dark brownish yellow in colour. Several small Graafian follicles bulge on the surface of section.

FIG. 20, *a*, *b*, *c*, *d*.

Merino ewe, No. 18; date, 26.2.30, 10 a.m.

Duration of last interoestrous period, 13 days.

Duration of last oestrus, 72 hours.

Duration of present oestrus, 18 hours.

The ewe was served twice by the teaser at the first appearance of oestrus, and once 4 hours prior to slaughter.

Left Ovary, Figs. *a* and *b*—

Measurements, 1.8×1.35×1.0 cm. Weight, 1.3 gm.

Fig. *a*.—Ovulation has not yet occurred. The ripe Graafian follicle measures 0.8 cm. in diameter. Its capsule is thin and transparent. A few small blood-vessels run across the follicular capsule. Some follicles of varying size are also present.

Fig. *b*.—Section is made through the ripe Graafian follicle. Some smaller follicles bulge on the surface of section.

Right Ovary, Figs. *c* and *d*—

Measurements, 1.6×1.5×1.1 cm. Weight, 1.2 gm.

Fig. *c*.—The corpus luteum I gives the ovary a somewhat cone-shaped appearance. It is distinctly visible through the surface. The capsule of ovarian tissue covering the corpus presents several small tortuous blood-vessels. There are several small Graafian follicles also present.

Fig. *d*.—Section shows the corpus luteum I, which measures 0.91×0.85 cm. somewhat oval in outline. It is very pale flesh-coloured with a distinct yellowish tinge. In its centre is a small rounded cavity, 0.15 mm. in diameter, which is filled with fluid. Some small Graafian follicles are also present.

FIG. 21, *a*, *b*, *c*, *d*.

Merino ewe, No. 33; date, 25.2.30, 2 p.m.

Duration of last interoestrous period, 14½ days.

Duration of last oestrus, 48 hours.

Duration of present oestrus, 26 hours.

The ewe was served by the teaser at the commencement of oestrus and again immediately before slaughter.

Left Ovary, Figs. *a* and *b*—

Measurements, 1.5×1.1×0.95 cm. Weight, 0.88 gm.

Fig. *a*.—Ovulation has not yet occurred. The ripe Graafian follicle measures 0.9 cm. in diameter. The capsule is thin and transparent in parts. In other parts the capsule is thin with a greyish tinge. Some smaller follicles of varying size are also evident through the surface.

Fig. *b*.—Shows section through the mature follicle. The remnants of corpus luteum II can be seen as a brownish irregular area.

Right Ovary, Figs. *c* and *d*—

Measurements, 1.65×1.0×1.0 cm. Weight, 0.9 gm.

Fig. *c*.—The corpus luteum I occupies the whole of the anterior pole of the ovary. It is just visible through the surface. Some tortuous vessels can also be seen. There are some small Graafian follicles present.

Fig. *d*.—Shows a section through the corpus luteum I. It measures 0.75×0.7 cm. It is somewhat triangular in outline and of a pale yellow colour. Some smaller follicles are also present.

OESTRUS

INTER-OESTRUS PERIOD

Chart showing the physiological changes in both ovaries of the Merino sheep during the sexual cycle

1
Fo. Gr. rpt. (= Co. lut. I.)
d
c

2
Co. lut II
Co. lut I
d
c

3
Co. lut I
d
c

4
Co. lut II
Co. lut I
d
c

5
Co. lut I
Co. lut II
b
a

6
Fo. Gr.
Co. lut I
Fo. Gr.
d
c

7
Co. lut I
b
Co. lut II
a

8
Co. lut I
c
Co. lut II
d

9
Co. lut I
a
b
Fo. Gr.
Co. lut II

10
Co. lut I,
c
d

11
Co. lut II
Co. lut I
b

12
a
Co. lut I
Co. lut II
b

13
Co. lut I
Co. lut II
d

14
c
Co. lut I
d

15
Co. lut I,
b

16
Fo. Gr. dil
Fo. Gr.
Co. lut I
b
Fo. Gr. dil

17
Fo. Gr. dil
14 days
a
b
Co. lut I
c
d

18
Co. lut I
a
Fo. Gr.
b

19
Fo. Gr. mt
Co. lut I
a
Fo. Gr. mt
b

20
d
Co. lut I
c

21
d
Co. lut I
c

Fo. Gr.
Co. lut II
b
a
36 hrs (ovulation)

Co. lut II
b
Fo. Gr. mt
a
26 hrs

b
a
Fo. Gr. mt.
18 hrs

Co. lut II
d
6 hrs
c

b
Co. lut II
12 hrs
a

b
a
41 hrs

Co. lut II
Fo. Gr.
b
1 day
a

Co. lut. r.
Fo. Gr.
Co. lut r.
d
1½ days
c

Co. lut II
3 days
b
Co. lut r.
a

Fo. Gr.
4 days
Co. lut I
d
c

Fo. Gr.
5 days
a
b

Fo. Gr.
6 days
c
d

7 days
a
Co. lut I,
b

8 days
c
Fo. Gr.

9 days
c
Fo. Gr.
d

10 days
a
Fo. Gr.
b

11 days
Co. lut II
a
Co. lut r.
b

12 days
Fo. Gr. dil
c
Co. lut II
d

13 days
c
Co. lut II
d
Fo. Gr.

14 days
Fo. Gr. dil
a
b

15 days to 18 days
Fo. Gr. mt.
c
d

del. C. G. Walker

(c) APPENDIX II.

The Changes in the Ovary during Pregnancy and Lactation.

This appendix shows in a series of coloured drawings the appearance of the ovary at intervals from the time of ovulation until parturition (Fig. 1 to 7). The ovarian changes during the early weeks of lactation are also illustrated (Figs. 8 to 12).

A full description of each figure is also given with details of the ewes and the weights and measurements of the ovaries.

Fig. 1, *a*, *b*, *c*, *d*.

Merino ewe, No. 30; date, 27.2.30, 9.30 a.m.

Duration of last interoestrous period, 14½ days.

Duration of last oestrus, 48 hours.

Duration of present oestrus, 40 hours.

During the present oestrus the ewe had been served once by the teaser at the commencement, once at the 24th hour, and once again at the 36th hour.

Left Ovary, Figs. a and *b*—

Measurements, 1.5×1.25×1.0 cm. Weight, 0.85 gm.

Fig. *a*.—The surface of the ovary shows several developing follicles of varying size.

Fig. *b*.—Section shows some small developing follicles bulging on the surface.

Right Ovary, Figs. *c* and *d*—

Measurements, 1.75×1.15×1.1 cm. Weight, 1.35 gm.

Fig. *c*.—Ovulation has already occurred. It is impossible to say when actual rupture took place, but it was a very short time previous to slaughter, as the blood around the point of rupture was not an organized clot. The point of rupture is raised somewhat above the surface of the surrounding follicular capsule. The actual ruptured area is circular and 0.2 mm. in diameter. Its border is irregular and saturated with blood, so that it forms a reddish circle. The follicular fluid (liquor folliculi) does not appear to have entirely escaped, as the cavity has not completely collapsed. The capsule around the rupture is yet transparent, so that the extent of the follicle is still recognizable. The corpus luteum of the previous interovulation period is present, just visible on the surface of the ovary as a whitish yellow elevation. Some Graafian follicles, varying in size, are also visible through the surface.

Fig. *d*.—Section shows the corpus luteum II (that of the completed inter-ovulation period), and the recently ruptured follicle. The follicular wall has become thickened, 0.25 cm. It is almost white in colour and soft, but does not break down like a clot when raised with a mounting needle. There is a small amount of colourless fluid present in the cavity, but no clotted blood. The cavity measures 0.3 cm. in diameter. The only blood coagulum present is attached to the borders of the actual rupture in the follicular wall.

The corpus luteum of the previous interovulation period has undergone rapid change since the commencement of oestrus. It has become much smaller, 0.55 cm., and has changed in colour to very pale yellow.

Fig. 2, *a*, *b*, *c*, *d*.

Merino ewe, No. 13; date, 6.5.30, 10 a.m.

Duration of gestation, 31 days.

Single pregnancy, male foetus in right horn.

Left Ovary, Figs. *a* and *b*—

Measurements, 1.6×1.3×0.75 cm. Weight 1.28 gm.

Fig. *a*.—Shows the surface of the ovary. There is a large Graafian follicle present. Its diameter measures 0.6 cm. Some smaller follicles are also present. Two dark brown pin-point specks, the remnants of corpora lutea of previous interovulation periods are evident through the surface.

Fig. *b*.—Shows the ovary on section. The large follicle described under Fig. *a* has been sectioned. The corpus luteum r. is seen. It is brownish, rounded, and measures 0.3 cm. in diameter.

Right Ovary, Figs. *c* and *d*—

Measurements, 2×1.5×1.2 cm. Weight, 1.45 gm.

Fig. *c*.—The corpus luteum vera is present. It projects somewhat above the surface of the ovary as a flat disc-like prominence. The presence of the corpus gives the ovary a slightly cone-shaped appearance, the apex of the cone being represented by the protruding portion of the corpus. A few Graafian follicles are present, two reaching a diameter of 0.45 and 0.4 cm.

Fig. *d*.—Shows the ovary on section. The corpus luteum vera is oval in shape. It measures 1.4×1.0 cm. It is muscle-colonred. A Graafian follicle has also been sectioned.

FIG. 3, *a*, *b*, *c*, *d*.

Merino ewe, No. (M.B.A.) 54; date, 26.2.30, 2 p.m.

Duration of gestation, 62 days.

Twin pregnancy, male foetus right horn, female foetus left horn.

Left Ovary, Figs. *a* and *b*—

Measurements, 1.65×1.65×1.1 cm. Weight, 1.28 gm.

Fig. *a*.—A corpus luteum vera, 1.0 cm. in diameter, is present. The ovarian capsule covering the corpus shows some tortuous blood-vessels. The corpus is not prominent, but forms a rounded extremity to the ovary. There are several small follicles of varying size present.

Fig. *b*.—Shows a section of the corpus luteum vera. It is muscle-coloured; about the same colour as raw beef. It measures 1.0 cm. in diameter.

Right Ovary, Figs. *c* and *d*—

Measurements, 1.9×1.3×1.1 cm. Weight, 1.61 gm.

Fig. *c*.—This ovary is similar to the left described in Fig. *a*. The diameter of the corpus luteum vera is also 1.0 cm.

Fig. *d*.—Section of this ovary is similar to the section of the left ovary described in Fig. *b*.

FIG. 4, *a*, *b*, *c*, *d*.

Merino ewe, No. (M.B.A.) 22; date, 26.2.30, 9 a.m.

Duration of gestation, 93 days.

Single pregnancy, male foetus right horn.

Left Ovary, Figs. *a* and *b*—

Measurements, 1.8×0.7×0.6 cm. Weight, 0.43 gm.

Fig. *a*.—The ovary is relatively small and light. Several Graafian follicles of small size are present. The largest follicle is about 0.25 cm. in diameter.

Fig. *b*.—Shows a section through the ovary. Towards the surface some small Graafian follicles bulge on the surface of section.

Right ovary, Figs. *c* and *d*—

Measurements, 2.1×1.2×1.0 cm. Weight, 1.12 gm.

Fig. *c*.—The corpus luteum vera is present. It measures 1.15 cm. in diameter. The ovarian capsule covering the corpus presents a purple or mauve colour. Some tortuous blood-vessels can be seen in the capsule. There are several small Graafian follicles present.

Fig. *d*.—Shows a section through the corpus luteum vera. It measures 1.15×1.1 in diameter. It is almost circular on section. It presents a dark red colour. In the centre there is a small cavity which has failed to become filled by the ingrowth of the corpus. One small Graafian follicle bulges on the surface of section.

FIG. 5, *a*, *b*, *c*, *d*.

Merino ewe, No. 1; date, 7.5.30, 9 a.m.

Duration of gestation, 116 days.

Single pregnancy, female foetus in right horn.

Left Ovary, Figs. *a* and *b*—

Measurements, 1.4×0.9×0.65 cm. Weight, 0.45 gm.

Fig. *a*.—The ovary is small and light. Its surface presents a few small Graafian follicles. Two remnants of corpora lutea from previous interovulation periods are present.

Fig. *b*.—Shows a section through the ovary. Two corpora lutea II can be seen as brownish somewhat irregular areas, surrounded by a whitish capsule.

Right ovary, Figs. *c* and *d*—

Measurements, 1.5×1.3×1.2 cm. Weight, 1.15 gm.

Fig. *c*.—The corpus luteum vera is present. It has become embedded so that the ovary has taken on a rounded appearance. It is possible, however, to detect its presence through the capsule, which appears mauve in colour. Evidence that the ovary tissue is closing over the corpus is demonstrated by the presence of a few small Graafian follicles lying superficial to it. Some small Graafian follicles are also present on the surface of the ovary close to the attachment of the meso-ovarium.

Fig. *d*.—Shows a section through the corpus luteum. It measures 0.85×0.85 cm. It is similar in colour to freshly cut beef muscle. The corpus luteum II is seen as a brownish speck. A distinct greyish-white capsule of ovarian tissue can be seen covering the surface of the corpus luteum vera.

FIG. 6, *a*, *b*, *c*, *d*.

Merino ewe, No. 27; date, 8.5.30, 9 a.m.

Duration of gestation, 149 days.

Single pregnancy, male foetus in left horn.

Left Ovary, Figs. *a* and *b*—

Measurements, 1.95×1.2×1.1 cm. Weight, 0.965 gm.

Fig. *a*.—The corpus luteum vera is present. It has become embedded, but its presence is easily recognizable through the ovarian capsule. It appears through the surface as mauve colour. A few small Graafian follicles are present in the ovarian capsule which covers the corpus. Some small follicles are also present on the surface of the remaining ovarian tissue.

Fig. *b*.—Shows a section through the ovary. The corpus luteum vera measures 0.9×0.8 cm. It is reddish brown in colour; a marked change in colour is noticed from the fresh muscle appearance of younger corpora lutea vera. The corpus luteum of the last interovulation period appears as a small brownish speck.

Right Ovary, Figs. *c* and *d*—

Measurements, 1.85×1.0×0.55 cm. Weight, 0.6 gm.

Fig. *c*.—The ovary is small and laterally flattened. Its surface shows numerous small Graafian follicles, the largest of which is 0.25 cm. in diameter.

Fig. *d*.—Shows the ovary on section. A few small Graafian follicles are present on the surface of section.

FIG. 7, *a*, *b*, *c*, *d*.

Merino ewe, No. 16; date, 10.5.30, 11.30 a.m.

The sheep was slaughtered at the moment of delivery of a female foetus.

Left Ovary, Figs. *a* and *b*—

Measurements, 1.5×0.95×0.55 cm. Weight, 0.45 gm.

Fig. *a*.—The ovary is small and flattened from side to side. The surface shows some small Graafian follicles.

Fig. *b*.—Shows a section through the ovary. Some small Graafian follicles are present.

Right Ovary, Figs. *c* and *d*—

Measurements, 1.55×1.25×0.8 cm. Weight, 0.65 gm.

Fig. *c*.—Shows the surface of the ovary with the regressing corpus luteum vera very slightly projecting. The projection is brownish yellow in colour. Some small Graafian follicles are also present.

Fig. *d*.—Shows a section through the ovary. The corpus luteum vera is yellowish brown in colour. It is almost entirely embedded. It measures 0.8×0.8 cm.

FIG. 8, *a*, *b*, *c*, *d*.

Merino ewe, No. 72; date, 10.5.30, 9 a.m.

Period since parturition, 5 days. The ewe suckled a male lamb.

Left Ovary, Figs. *a* and *b*—

Measurements, 1.5×1.0×0.55 cm. Weight, 0.49 gm.

Fig. *a*.—The ovary is small and laterally flattened. The surface shows some small Graafian follicles. One fairly large follicle, measuring 0.5 cm. is present.

Fig. *b*.—Shows the ovary on section. Some small Graafian follicles are present on the surface of section.

Right Ovary, Figs. *c* and *d*—

Measurements, 1.65×1.2×0.75 cm. Weight, 0.75 gm.

Fig. *c*.—The regressing corpus luteum vera is present, but is only just visible through the surface, where the ovarian capsule covering it presents a whitish yellow tinge. Several small Graafian follicles are present, the largest is 0.5 cm. in diameter.

Fig. *d*.—Shows a section through the corpus luteum vera. It measures 0.6×0.6 cm. It presents a light brownish yellow colour. It has become entirely embedded. A Graafian follicle has also been sectioned.

FIG. 9, *a*, *b*, *c*, *d*.

Merino ewe, No. 6; date, 12.4.30, 10 a.m.

Period since parturition, 10 days. The ewe suckled an ewe lamb.

Left Ovary, Figs. *a* and *b*—

Measurements, 1.6×1.0×0.8 cm. Weight, 0.75 gm.

Fig. *a*.—Shows the surface of the ovary. There is a large developing Graafian follicle. It is confined to the posterior pole. It measures 0.6 cm. in diameter. Several smaller follicles are also present. The regressing corpus luteum vera is just visible through the surface as a light brownish speck.

Fig. *b*.—Shows a section through the corpus luteum vera. It is brownish yellow in colour and measures 0.45×0.45 cm. in diameter. The large Graafian follicle described in fig. *a* has also been cut through.

Right Ovary, Figs *c* and *d*—

Measurements, 1.5×1.1×0.75 cm. Weight, 0.61 gm.

Fig. *c*.—Two large developing Graafian follicles are present, measuring (1) 0.55 cm., (2) 0.5 cm. in diameter. Some smaller follicles are also present.

Fig. *d*.—Shows a section through the ovary. The two Graafian follicles described in fig. *c* have been sectioned.

FIG. 10, *a*, *b*, *c*, *d*.

Merino ewe, No. 41; date, 8.5.30, 2 p.m.

Period since parturition, 14 days. The ewe suckled a male lamb.

Left Ovary, Figs. *a* and *b*—

Measurements, 1.3×1.3×0.7 cm. Weight, 0.55 gm.

Fig. *a*.—The corpus luteum appears through the surface as a brownish area. There are some small Graafian follicles present. At one part of the surface there is a depressed area which proves to be a very recently ruptured follicle. No haemorrhage is seen around the ruptured border.

Fig. *b*.—Shows a section through the regressing corpus luteum vera and the ruptured follicle. The corpus luteum measures 0.5×0.4 cm. It is brownish in colour.

Right Ovary, Figs. *c* and *d*—

Measurements, 1.2×1.0×0.65 cm. Weight, 0.5 gm.

Fig. *c*.—The surface of the ovary presents some small Graafian follicles, some of which stand out fairly prominently.

Fig. *d*.—Shows a section through the ovary. Some small Graafian follicles can be seen.

FIG. 11, *a*, *b*, *c*, *d*.

Merino ewe, No. 49; date, 9.5.30, 2 p.m.

Period since parturition, 12 days. The ewe suckled a male lamb.

Left Ovary, Figs. *a* and *b*—

Measurements, 1.5×1.45×0.9 cm. Weight, 0.78 gm.

Fig. *a*.—Shows several small Graafian follicles on the surface of the ovary.

Fig. *b*.—Shows a section through the regressing corpus luteum vera. It measures 0.45×0.4 cm. It is brownish yellow in colour.

Right Ovary, Figs. *c* and *d*—

Measurements, 1.8×1.3×0.95 cm. Weight, 1.37 gm.

Fig. *c*.—The corpus luteum I is present. It protrudes above the surface of the ovary as a rounded flattened prominence 0.4 cm. in diameter. There is a large Graafian follicle also present. It measures 0.55 cm.

Fig. *d*.—Shows a section through the corpus luteum I. It measures 0.85×0.75 cm. It contains a cyst-like cavity 0.5×0.4 cm. The cavity contained a clear fluid. The corpus is pale pink in colour. A large Graafian follicle has been sectioned; another uncut follicle also appears on the surface of section.

FIG. 12, *a*, *b*, *c*, *d*.

Merino ewe, No. 11; date, 9.5.30, 9 a.m.

Period since parturition, 25 days. The ewe suckled a male lamb.

Left Ovary, Figs. *a* and *b*—

Measurements, 1.25×0.85×0.65 cm. Weight, 0.45 gm.

Fig. *a*.—Shows the surface with a Graafian follicle measuring 0.6 cm. in diameter. Traces of the remnants of previous corpora lutea can be seen.

Fig. *b*.—Shows a section through the ovary. The large Graafian follicle described in fig. *a* has been sectioned.

Right Ovary, Figs. *c* and *d*—

Measurements, 1.85×1.25×1.0 cm. Weight not recorded.

Fig. *c*.—There is a corpus luteum I present, which projects somewhat above the surface of the ovary as a flattened nipple-shaped prominence, which measures 0.4 cm. in diameter. The prominence is yellowish red in colour. It is not distinctly marked off from the ovarian surface. Two developing Graafian follicles are also present, each measuring 0.6 cm. in diameter. One is present at either pole of the ovary. Some smaller Graafian follicles are also present.

Fig. *d*.—Shows a section through the corpus luteum I. It measures 1.1×0.95 cm. It is a pale pink in colour. A small Graafian follicle is also seen on the surface of section.

Chart showing the physiological changes in both ovaries of the merino sheep during pregnancy and early lactation

PERIOD OF GESTATION

EARLY LACTATION 10-14 days after birth

PREGNANCY

1 — Co. luf. I; Co. luf. I; Fo. Gr.; Ovulation

2 — Co. luf. I. = Co. luf. V.; Co. luf. r.; 1 Month

3 — Co. luf. V; Co. luf. V; 2 Months

4 — 3 Months; Co. luf. V.; Co. luf. V.

5 — 4 Months; Co. luf. r.; Co. luf. r.; Co. luf. V.; Co. luf. II

6 — 5 Months; Co. luf. V; Co. luf. II

7 — At Birth; Fo. Gr.; Co. luf. V.

8 — 5 Days after Birth; Co. luf. V. r.; Fo. Gr. dil.

9 — Fo. Gr. mk.; Co. luf. V. r.; Fo. Gr. mk.; Fo. Gr. mk.

10 — Co. luf. V. r.; Fo. Gr. rpl. = Co. luf. I

11 — Co. luf. V. r.

12 — Fo. Gr.; Fo. Gr.

25 Days after Birth — Co. luf. I; Co. luf. I; Fo. Gr.

Del. C. G. Walker

Section VIII.

Poisonous Plants.

D. G. Steyn ... ... Recent Investigations into the Toxicity of known and unknown Poisonous Plants in the Union of South Africa.

D. G. Steyn ... ... Investigations into the cause of Alopecia (Kaalsiekte) in Kids and Lambs.

17th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1931.

Recent Investigations into the Toxicity of Known and Unknown Poisonous Plants in the Union of South Africa.

(Continued from 15th Report of D.V.S., 1929.)

By D. G. STEYN, B.Sc., Dr.Med.Vet., Veterinary Research Officer, Onderstepoort.

AIZOACEAE.

Mesembrianthemum anatomicum Harv.

Common names: Kou-goed; Kanna.

Origin: M. J. Ferreira, Skildpadbeen, Willowmore.

Stage of development: In flowering stage.

One rabbit received 35.0 grams of the fresh plant (leaves and flowers) *per os*.

Result: No symptoms of poisoning developed.

A few specimens of this plant were planted out on a rockery in the poisonous plant garden at Onderstepoort. After a period of two months the plant was again tested on rabbits.

One rabbit received 160 grams of the fresh leaves of the plant, in the pre-flowering stage, *per os*.

Result: Animal remained healthy. The fresh plant contains a large amount of water, so that it was unnecessary to add water in order to render the plant fit for dosing.

Literature: Pammel (1911) quotes *Mesembrianthemum anatomicum* Harv. as a narcotic. Marloth quotes it under the common name of " kanna ", whereas the plant is known as " kou-goed " by the coloured and European people in Willowmore. A few drops of fluid squeezed out of the leaves of this plant is used as a sedative by the coloured people. Experiments have shown that rabbits can be dosed with 160 grams of the plant with impunity.

Mesembrianthemum Mahoni N.E. Br. is mentioned by Burtt-Davy (1912) as being utilised by natives in the preparation of a fermenting agent for the manufacturing of a drink called " Khadi " and as a rising principle for bread. For these purposes the natives use the residue of an evaporated watery extract of the debarked roots.

As it was maintained that in the course of time this preparation exerts toxic effects on its consumers, a quantity of the dried roots were forwarded to the Imperial Institute for chemical investigation. It was ascertained that the roots contained 3 per cent. of oxalates and it is held that constant consumption of the extract might cause oxalate poisoning.

Marloth (1917) records *Mesembrianthemum tortuosum* L. as "kauwgoed" and Meiring mentions this plant as being used as a soporific, one or two drops of the juice of the green plant producing a deep, quiet rest for a few hours in children. This effect Meiring (1895) ascribed to the decided alkaline nature of the juice, which is so great that the juice is used for washing clothing when soap is unobtainable.

Meiring (1895) quotes from Pappe. Flor. Cap. Med. Prodroms. 1868: "This species (*Mesembrianthemum tortuosum* L.), a native of the Karroo, appears to possess narcotic properties. The Hottentots, who know it by the name of 'kou-goed', are in the habit of chewing it, and become intoxicated. The farmers use it, in the form of a decoction or tincture, as a good sedative."

From this plant Meiring isolated an alkaloid, which on subcutaneous injection into frogs produced hurried breathing uneasiness, marked moisture of the skin and in some cases death. In guinea-pigs it produced uneasiness and inappetence, and. in one of two cases, death within twenty-four hours.

Psilocaulon sp. (Nat. Herb. No. 8819; Onderstepoort Herb. No. 42).

Mrs. Bolus, of Bolus Herbarium, is at present engaged upon the identification of this specimen.

Common names: Asbos, loogbos.

Origin: Mr. Strydom, Tweefontein, Willowmore.

While on an official visit to Willowmore, I was called to the above farm to investigate mortality in Angora goats. The affected animals exhibited cachexia, extreme weakness, anaemia, chronic diarrhoea and inappetence. Two to three weeks elapsed from the time the symptoms appeared until death occurred.

Death appeared to be due to debility. The post-mortem revealed anaemia, hydroperitoneum, hydrothorax, hyperaemia of the lungs, liver and kidneys, and marked irritation of the gastro-intestinal tract. No internal parasites were seen, and the owner assured me that he had not treated the animals.

I might state that at the time of my investigation serious drought conditions prevailed in that area.

Chemical investigation of the drinking water revealed no suspicious substances. When inspecting the veld on which the animals had been grazing the owner informed me that lately, owing to the drought, the animals had taken to eating the so-called "asbos" or "loogbos" as this plant formed practically the only "edible" succulent vegetation.

A small amount of the bush which was in the post-seeding stage and appeared withered, was collected and drenched to rabbits. Each of two rabbits received 50 grams of the wilted plant *per os*.

Result: Within half an hour the animals became restless, the pulse extremely accelerated and weak and the respiration hurried and superficial. The head was thrown backwards and death took place under most severe convulsions within one hour after dosage.

The post-mortem revealed a marked general cyanosis, pronounced hyperaemia of liver and lungs and kidneys, stomach distended with gas, and mucous membrane intensely dark reddish in colour and exhibiting numerous small haemorrhages.

A few specimens of this plant were planted out on the rockery in the Onderstepoort poisonous plant garden. Two months' subsequently luxurious growth had occurred, and it was decided again to test the plant.

Each of two rabbits received 120 grams of the fresh plant in the pre-flowering stage *per os*, with completely negative results.

The ashes of this plant are used in the preparation of lye for soap-making and the dipping of raisins and tobacco.

Discussion.—The goats in question appeared to die from debility caused by constant irritation of the gastro-intestinal tract. As no other source of the trouble could be found than the suspected " asbos " and as this bush, under the then existing drought conditions most decidedly caused severe irritation and rapid death when given in big doses, there appears to be little doubt that this bush was the cause of the trouble.

The fact that large amounts of the " asbos ", which had been brought from Willowmore and planted at Onderstepoort failed to cause death in rabbits, whereas much smaller amounts of the original bush proved to be fatal to these animals, suggests a variation in the toxicity of this plant grown under different climatic and tellurgic conditions.

Amaryllidaceae.

Amaryllis belladonna L.

Common names: Maart lelie, March lily, Amaryllis, Belladonna lily.

Experiments conducted with this plant have been published in the 15th Report of the Director of Veterinary Services, 1929, p. 779, under *Nerine* sp., probably *N. marginata*. This plant has now been definitely identified as *Amaryllis belladonna* L.

Pammel mentions that *Amaryllis belladonna* contains an alkaloid belladonin, but furnishes no details as to its toxic properties.

Isolation of the toxic principle(s).—The bulbs, when cut up and dried in the sun for a period of three months proved not to have decreased in toxicity. The undermentioned investigations were conducted with fresh and half-dried seeds.

The toxin was found to be most soluble in 96 per cent. alcohol, 50 per cent. acidulated alcohol and ether, and less soluble in distilled water and chloroform. 750 grams of the fresh and half-dried seeds were minced and extracted for twenty-four hours. This process was repeated once more. This evaporated extract proved to be extremely toxic both on subcutaneous injection into guinea-pigs and when dosed to rabbits.

To this extract was added basic lead acetate until precipitation was exhausted, the excess lead removed by adding dilute sulphuric acid, the filtrate partially neutralised with solid calcium carbonate. The filtrate was then made alkaline with caustic soda solution and shaken out with ether, which removed the toxin from the filtrate. This ethereal solution of the toxin was evaporated and left as residue a light yellowish-brown resinous substance, which proved to be extremely toxic on subcutaneous injection into guinea-pigs.

This residue was then taken up in a few cubic centimetres of distilled water slightly acidulated with dilute hydrochloric acid and then submitted to the following alkoloidal tests:—

(1) Sheibler's Reagent—thick white precipitate.

(2) Mayer's Reagent—thick white white precipitate.

(3) Wagner's Reagent—thick dark-brown precipitate.

(4) Hager's Reagent—thick yellow precipitate.

The seed residue of the absolute alcoholic extract after having been washed with absolute alcohol was repeatedly extracted with ether. The filtrate after evaporation proved to be non-toxic to guinea-pigs when injected subcutaneously.

After the seed had been extracted and washed with ether, it was repeatedly extracted with chloroform. After evaporation also this residue proved to be non-toxic to guinea-pigs.

Subsequently this seed was repeatedly extracted with distilled water with negative results.

Rabbits were dosed with large amounts of the above extracted seed with negative results.

Conclusion.—Mature *Amaryllis belladonna* seed contains a poisonous alkaloid(s), which is very soluble in absolute alcohol and 50 per cent. acidulated alcohol and ether, and to a lesser extent in chloroform and distilled water. It is possible to remove all the toxin contained in the seed by repeated extractions with absolute alcohol.

Haemanthus amarylloides Jacq.

Origin: Poisonous Plant Garden, Onderstepoort.

Sheep 28223: Received 500 grams of fresh bulbs and leaves of the above plant in the late seeding stage.

Result: Within one hour after dosage the animal exhibited pronounced salivation, an accelerated respiration, and an accelerated but strong pulse. The following morning the animal was found lying down, unwilling to rise, markedly bloated and groaning on expiration. There were profuse salivation, lachrymation and diarrhoea,

and the conjunctivae were intensely dark purplish in colour. The pulse was extremely accelerated and very weak, the respiration shallow, costo-abdominal and hurried, and the temperatnre subnormal. The animal died within twenty-four hours after dosage.

Post-mortem appearances: Marked general cyanosis with ecchymoses on the conjunctival mucous membranes; on skinning the animal a large amount of dark-reddish blood flowed from the subcutaneous tissues; numerous subendocardial and subepicardial haemorrages; pronounced hyperaemia of the lungs, liver, periportal, mediastinal and retropharyngeal lymph glands, with haemorrhages in the last named; severe acute haemorrhagic gastro-enteritis with numerous haemorrhages throughout the gastro-intestinal tract; degenerative changes in the liver.

This apparently is the first toxic record of this plant.

Haemanthus concolor Herb.

Origin: P. L. le Roux, Pokkraal, Worcester.

Sheep 26057: Received 500 grams of the fresh bulbs of the above plant in the late flowering and early fruiting stage.

Result: Completely negative.

Narcissus jonquilla Linn.

Origin: Somerset East.

One rabbit received 50 grams of the bulbs (with no flowers, and leaves just appearing) *per os*.

Result: The following morning the animal appeared listless and exhibited accelerated breathing, an accelerated but good pulse, and inappetence. It died with symptoms of asphyxia within twenty-four hours after dosage.

Post-mortem appearances: General cyanosis; hyperaemia of the lungs; both ventricles markedly dilated and distended with coagulated blood; degenerative changes in the liver; multiple localised hyperaemia of the gastric mucosa.

Nerine lucida Herb.

Origin: Mr. Hauptfleisch, Dam, P.O. Springfield, Griqualand West.

The owner suspected that this plant has in the past been the cause of mortality in his goats as he has seen them eating the plant.

Sheep 27977: Received 200 grams of the fresh bulbs (without leaves and flowers) *per os* at 10 a.m. on 3.6.30.

Result: The following morning the animal was found prostrate and markedly bloated. The respiration was slow (10 per minute) and deep, the pulse was decreased in rate and extremely irregular. At varying intervals the animal stretched the legs and threw back the head. Clonic spasms of different parts of the body were to be seen. It died within twenty-four hours after dosage.

Post-mortem appearances: Slight general cyanosis; both ventricles of the heart markedly dilated and heart extremely flabby; slight hyperaemia of the lungs; pronounced hyperaemia and swelling of the spleen; rumen markedly distended with gas; abomasal mucosa appeared swollen.

No toxic records of this plant could be found in the literature at my disposal.

Araliaceae.

Cussonia spicata Thunb.

Common names: Kiepersol, nooisblom, sambreel, cabbage-tree.

Origin: Grahamstown. The leaves of this plant were supposed to have caused poisoning in cattle. Unfortunately only a small quantity of the leaves were forwarded.

Sheep 21100: Received 200 grams of the fresh leaves *per os*.

Result: Negative.

Asclepiadaceae.

Asclepias fruticosa L.

Common names: Gansies, melkbos, tondelbos, wilde kapok, firesticks, shrubby milkweed, wild cotton.

Origin: Onderstepoort.

Queries as to the toxicity of this plant for our domestic animals formed the basis of this experiment.

One rabbit received 40 grams of the fresh flowers, leaves and upper parts of the stems *per os*.

Result: Within one hour after dosage the animal appeared restless, and exhibited weakness of the neck, being unable to keep the head up. The pulse was extremely accelerated, the respiration deep, and costo-abdominal. Within another half an hour the animal was unable to raise its head. The respiration became increasingly laboured until the animal breathed with the open mouth. The heart-beat became irregular and weak. Ultimately the animal struggled and gasped for breath, until the respiration ceased. The heart-beat continued for about two minutes after the respiration had stopped. While the heart was still beating faintly, there were fibrillary contractions of different groups of muscles, especially those of the back and abdomen.

Post-mortem appearances: Marked hyperaemia of the lungs; both ventricles of the heart dilated.

No toxic records of this plant could be found in the available literature.

Sarcostemma viminale R. Br.

Common names: Melktou, spantou, melkbos.

Origin: Mr. Landrey, Kei Road, Cape Province.

Sheep 28412: Received 1,100 grams of the fresh vines (without fruit or flowers) on two consecutive days.

Result: Negative.

Capparidaceae.

Capparis albitruncata Burch.

Common names: Witstam, witgatboom, shepherdstree.

Origin: Griqualand West.

In Griqualand West and other parts of South Africa the roots of this tree are being extensively used in the preparation of " coffee ". It is maintained that this " coffee ", if taken by anybody not accustomed to it, causes gastro-intestinal disturbances. Consequently, it was thought that the roots contain some or other injurious ingredient. A quantity of the roots were obtained for experimental purposes.

The root has a peculiar structure, cylindrical wooden " rootlets " being embedded in a soft, pulpy mass. This pulpy part is utilised in the preparation of the " coffee ", whereas the hard, wooden part is discarded.

One rabbit received 220 grams of the fresh pulpy part of the root on three consecutive days without any deleterious effects.

Compositae.

Aster filifolius Vent.

Origin: Grahamstown.

This plant was suspected to have caused mortality in sheep.

Sheep 25638: Received 350 grams of the fresh stems, leaves and flowers.

Result: The animal was found dead the next morning without any symptoms having been noticed.

Post-mortem appearances: Pronounced general cyanosis; blood partly coagulated and intensely dark-reddish in colour; subcutaneous blood vessels markedly distended; hydroperitoneum; hydrothorax; oedema and hyperaemia of the lungs; numerous subendorcardial haemorrhages in both ventricles; marked tumor splenis, with pronounced hyperaemia and malpighian bodies very prominent; venous congestion of the liver; hyperaemia of the retropharyngeal lymph glands.

Sheep 27988: Treated as follows with dried flowers, leaves and upper parts of the stems of the flowering plant: 15.9.30, 100 grams *per os*.

16.9.30: As animal appeared normal another 100 grams were given at 12 noon. At 2 p.m. the animal stood with back arched, appeared listless, groaned on expiration, was slightly tympanitic, the pulse accelerated and strong, respiration hurried and costo-abdominal. At 6 p.m. its condition was much worse, the animal exhibiting marked salivation, tympanites and an accelerated pulse.

17.9.30: Condition much improved. Another 100 grams given with result as described above.

18.9.30: Condition improved. Another 100 grams given. Again transitory symptoms of poisoning occurred.

19.9.30: Condition improved. Another 100 grams given. Again transitory symptoms of poisoning occurred.

20.9.30: Animal appears fairly ill; respirations short, superficial and accelerated; pulse accelerated and not strong, pronounced salivation and white froth appearing on lips, diarrhoea, groaning on expiration.

21.9.30: Died during the previous night.

Post-mortem appearances: In addition to the lesions described in sheep 5638, this animal showed the presence of ruminal contents in the main bronchi and trachea; a pronounced acute catarrhal gastro-enteritis with numerous haemorrhages in the mucosa.

Sheep 28266: Received 300 grams of flowers, leaves and stems of the dried plant.

Result: Within four hours the animal became tympanitic and the pulse and respiration were accelerated. A progressive weakness set in until seven hours after dosage the animal was unable to rise. The pulse became imperceptible in the course of another two hours, and the animal died during the course of the following night.

Post-mortem appearances: General cyanosis; blood intensely dark-reddish in colour; hydroperitoneum; hydrothorax; oedema of the lungs; numerous subendocardial haemorrhages both ventricles; pronounced venous congestion of the liver; slight acute catarrhal abomasitis and jejunitis; hyperaemia of the retropharyngeal lymph glands, and oedema of the mediastinal lymph glands.

Geigeria pectidea Harv.

Origin: Mr. Hauptfleisch, Dam, P.O. Springfield, Griqualand West.

Sheep 21778: Received the following amounts of leaves, stems, flowers and seeds of the dry plant in the late flowering and seeding stage.

28.5.30: 300 grams at 12 noon; 350 grams at 3 p.m. At 4 p.m. the animal exhibited uneasiness, marked salivation, tympanites, accelerated respiration and pulse.

29.5.30: 350 grams. Condition as above and greenish diarrhoea had developed.

30.5.30: 350 grams. The above symptoms had increased in severity. The pulse was extremely accelerated and weak. Furthermore, there were pronounced tympanites, retching, vomiting, and a stiff gait, which was so marked that the animal staggered about when driven. Death occurred at 12 noon.

Post-mortem appearances: Rumen markedly distended with gas; hyperaemia and slight oedema of the lungs; degeneration of the myocard, liver and kidneys; hyperaemia of and haemorrhages in the retropharyngeal lymph glands; an acute catarrhal duodenitis and jejunitis.

Senecio bupleuroides D.C.

Origin: Mr. Struben, Fairy Glen, Pretoria.

Sheep 21778: Received 1,200 grams leaves and stems of the dry plant in the post-seeding stage on four consecutive days.

Result: Negative.

Senecio glutinosus Thunb.

Origin: Bathurst Experiment Farm, Grahamstown.

Sheep 28233: Received 1,000 grams wilted and mouldy leaves, stems and flowers on two consecutive days.

Result: Negative.

Senecio isatideus D.C.

Common names: Dan's Cabbage.

Origin: Mr. Mossop, Cliffdale, Hlobane, Natal.

EXPERIMENT I.

Horse 19226 (six months old) was fed 2,740 grams of dried *Senecio isatideus* and 810 grams of dried *Senecio latifolius* in the course of ten months. The animal developed symptoms resembling very closely those of "Dunsiekte." A detailed description of this case will be found in the article on Dunsiekte by du Toit, de Kock and Steyn, published elsewhere in this report.

EXPERIMENT II.

To ascertain whether *Senecio isatideus* had decreased in toxicity after it had been stored for sixteen months.

Sheep 26087: Received 80 grams dried leaves of the flowering plant at 12 noon on the 28.3.30.

Result: 29.3.30, 9 a.m., listless, lying down, unwilling to rise, accelerated respiration and pulse, and not feeding.

30.3.30: Condition worse, conjunctivae yellowish.

31.3.30: Died at 2 p.m.

Post-mortem appearances: Anaemia; slight general icterus; slight oedema of the subcutaneous connective tissues; oedema of the lungs; tumor splenis with hyperplasia of the malpighian bodies; extensive regressive changes and hyperaemia of the liver; fatty changes of the kidneys; subendocardial and subepicardial haemorrhages; acute catarrhal gastro-enteritis with pronounced oedema of the leaves of the abomasum; oedema of the perirectal tissues.

EXPERIMENT III.

To ascertain whether the *Senecio isatideus* is poisonous in the the late seeding stage.

Unfortunately the plant arrived at Onderstepoort in a decomposed state.

Sheep 26118: Received 150 grams of above dry plant at 12.45 p.m. on 28.5.30.

Result: 29.5.30, no symptoms appeared.

30.5.30, no symptoms appeared. Another 100 grams.

31.5.30-4.6.30, no symptoms developed.

4.6.30, another 200 grams.

Until the evening of 6.6.30 the animal appeared perfectly normal. At 8 a.m. on 7.6.30 it was found pressing its head with the force of whole body into a corner of the stable (see Fig. I). The animal was placed near a tree and immediately commenced pushing. The back was arched and the body swaying from side to side. The breathing was stertorous and extremely laboured and the temperature normal. The pulse counted 176 per minute and was weak, and the conjunctivae showed a decided yellowish tinge. When forced to walk the animal staggered about with out-spread legs. The pulse became progressively weaker and white froth appeared at the mouth. Death supervened at 11 a.m. on the same day.

Post-mortem appearances: Slight general icterus; hydroperitoneum; hydrothorax; oedema of the bronchial and mediastinal lymph glands; hyperaemia of the spleen; hyperaemia and regressive changes in the liver; acute catarrhal cholecystitis; haemorrhage in and ulceration of the ruminal mucosa; croupous duodenitis and jejunitis; oedema of the perirectal tissues.

Ninety grams of dried leaves of the plant in the flowering stage caused death in a sheep within three days after dosage, whereas 250 grams of the dried leaves of the plant in the late seeding stage failed to produce symptoms of poisoning within the course of seven days. An additional 200 grams of the plant proved to be fatal.

No definite conclusions as to the degree of toxicity of the plant in the late seeding stage can be drawn as the process of decomposition might have destroyed part of the toxin.

There was no decrease in the toxicity of *Senecia isatideus* after it had been stored for sixteen months.

Senecio othonnaeflorus D.C.

Common name: Bietou.

Origin: Humansdorp.

This plant was forwarded to Onderstepoort with the remark that it caused tympanites and death in cattle. It will be noticed that the plant is commonly known as "bietou," a name which is applied mostly to *Dimorphotheca* species.

Only a small quantity of the plant was forwarded so that the test had to be limited to one rabbit.

One rabbit: Received 50 grams of fresh stems, leaves and flowers of the plant given *per os*.

Result: Negative.

Fig. 1.—Sheep 26118 in the act of pushing against a tree. Photographed one hour before death.

Senecio pinnulatus Thunb.

Origin: Bathurst Experiment Station, Grahamstown.

The plant arrived at Onderstepoort in a mouldy state.

Sheep 29192: Received 1,000 grams of leaves, stems and flowers of the above plant on two consecutive days.

Result: Negative.

CRASSULACEAE.

Cotyledon pillansii Schonl.

Origin: M. J. Ferreira, Skildpadbeen, Willowmore.

One rabbit: Received 100 grams of fresh leaves of the above flowering plant *per os*.

Result: Negative.

In addition a 96 per cent. alcoholic extract of the fresh leaves was prepared and the evaporated extract injected subcutaneously into guinea-pigs.

1 guinea pig: 0.5 c.c. (= 10 grams fresh leaves).

1 guinea-pig: 1.5 c.c. (= 30 grams fresh leaves).

1 guinea-pig: 5.0 c.c. (=100 grams fresh leaves).

Result: All animals remained healthy.

Cotyledon ventricosa Burm.

Common names: Xenta, Krimpsiektebossie.

Origin: Springbok, Namaqualand.

One rabbit: Received 40 grams of slightly wilted leaves of the plant in the pre-flowering stage.

Result: Laboured breathing set in within one hour after dosage. General weakness developed and progressed until the animal was unable to sit up. Spasms of the diaphragm and different groups of body muscles occurred and death under symptoms of asphyxia supervened five hours after dosage.

Post-mortem appearances: General cyanosis; diaphragm appeared to be in a state of spasm.

EUPHORBIACEAE.

Acalypha punctata Meisn.

Origin: L. J. Nel, Driepoort, Carolina.

Sheep 21778: Received 80 grams of leaves and stems of the dried plant in the pre-flowering stage.

Result: Negative.

In America *Acalypha colchica*, Fisch. and Mey, is reported to contain prussic acid, while other species of *Acalypha* are emetics, expectorants, diuretics and irritants.

Euphorbia helioscopia Linn.

Common name: Melkbos.

Origin: Capetown.

This plant was suspected of having caused mortality in stock.

Sheep 28437: Received the following amounts of fresh stems, leaves, flowers, green and ripe fruits of the plant:—

9.10.30.—850 grams.
11.10.30.—500 grams.
13.10.30.—400 grams.

Result: Negative.

GERANIACEAE.

Monsonia ovata Cav.

Common names: Keita, naaldbossie, dysentery herb.

Origin: East London.

An "extract" of this plant which is being sold as a remedy for calf dysentery was submitted for investigation as to its toxicity.

One rabbit: Received 100 c.c. of the "extract" *per os*.

Result: Negative.

MacOwen (1877), reporting on *Monsonia biflora* D.C. and *Monsonia ovata* Cav., wrote both plants were used by the Hottentots in dysentery and diarrhoea. A decoction of two-thirds of an ounce of the plant in one pint of water is prepared and a tablespoonful taken six to eight times a day. These plants are said to contain tannin. Furthermore, the juice of a species of *Monsonia* growing in the Transvaal and Rhodesia is squeezed on to anthrax pustules.

GRAMINAE.

Arundo donax Linn.

Common name: Spanish reed.

Origin: Boetsap, Griqualand West.

Sheep 28437: Received 600 grams of the fresh young reeds.

Result: Negative.

IRIDACEAE.

Moraea edulis Ker var. *longifolia Sweet*.

Common name: Uintjie.

Origin: P. L. le Roux, Pokkraal, Worcester. The plant had been stored at Onderstepoort for one year and fourteen days.

Sheep 20753: Received 90 grams of dried leaves and flowers of the above plant.

Result: Negative.

Moraea simulans Bkr.

Origin: Rustenburg.

Farmers maintain that this plant is most toxic when in flower.

One Rabbit: Received 10 grams of dried leaves and fruit.

Result: Negative.

Moraea viscaria Ker.

Common name: Teer Uintjie.

Origin: P. L. le Roux, Pokkraal, Worcester.

The plant had been stored at Onderstepoort for one year.

Sheep 19250: Received 250 grams of dried leaves and flowers of the above plant.

Result: Negative.

LEGUMINOSAE.

Canavalia ensiformis D.C.

Twenty-two kilograms of the ripe beans were forwarded to Onderstepoort by the Division of Plant Industry, Pretoria, with the request that feeding tests be conducted with cattle. In Rhodesia it is being used chiefly as a green manure, and in the East oxen are fed on the seeds, these being first broken up and boiled before being fed. In Hawaii the green bean has given good results in dairy cows.

Bovine 2792: The animal was unwilling to take the beans whether whole or ground, or, mixed with bran or green forage, but took readily any mixture of the beans to which molasses had been added.

The 22 kilograms were ingested in the course of twenty days.

Result: There was no evidence of the ripe beans having any deleterious effect on the health of the animal.

Cassia obovata Collad.

Common names: Elands-ertjie, elandspea, wild senna.

Origin: Matzap, Griqualand West.

This plant was alleged to have caused heavy mortality in goats.

Goat 22366: Received 450 grams of slightly wilted leaves, young stems and flowers on 28.11.29, and another 300 grams of the dried leaves on 29.11.29.

One rabbit: Received 15 grams of the slightly wilted and 15 grams of the dried plant on two consecutive days.

One rabbit: Received 7.5 grams of the slightly wilted and 7.5 grams of the dried plant on two consecutive days.

Result: All animals remained healthy.

Crotalaria allenii Verdoorn.

Origin: Angola Farmers' Settlement, South West Africa.

This species of *Crotalaria* was suspected to have caused stiff-sickness in cattle.

Sheep 24692: Received 10.5 kilograms of dried stems, leaves, flowers and seeds during a period of forty-two days.

Result: The animal suffered no ill-effects.

Bovine 2792: This animal refused to take the plant mixed with the ordinary food, consequently drenching by means of a stomach tube had to be resorted to. Small quantities (100 grams) were, however, also mixed with the food. The animal received 32.4 kilograms of the dried plant in the flowering and seeding stage during a period of two months.

Result: No symptoms of stiffsickness or any other ill-effects were developed.

Crotalaria distans Bth.

Origin: Onderstepoort.

Sheep 21778: Received 1,800 grams of fresh leaves, stems and flowers on two consecutive days.

Result: Negative.

Crotalaria dura Wood and Evans.

Origin: Allerton Research Laboratory, Pietermaritzburg, Natal.

To ascertain whether a period of five years' storage would have a detrimental effect on the toxicity of *Crotalaria dura*.

The plant was in the late seeding stage and had been stored at Onderstepoort for five years.

Horse 19662: Ingested approximately 42 kilograms of the dried stems, leaves and ripe seeds of the above plant during the course of two months.

In order to prevent the animal from inhaling dust the plant material was slightly moistened before being mixed with the daily ration. Great trouble was experienced in persuading the animal to take any foodstuff mixed with the plant.

Result: The first symptoms appeared six weeks after the inception of the experiments. The respiration was laboured, more of the abdominal type and the expiration stressed and double, the animal began feeding less and was apathetic. There was a definite fever from the sixty-fourth day up to the time of death, when the temperature measured 102.2° F., and a slight yellowish discoloration of the conjunctiva was discernible. Eight days later the colour of the conjunctiva had changed into a dirty yellowish-brown and ecchymoses were present, the pulse, which had remained strong although accelerated, was very weak and counted eighty-four per minute and the respiration one hundred and twenty, being of the abdominal type. The animal was swaying from side to side and exhibited a constant inclination to fall forward, but prevented the latter by supporting its head on the manger. It was standing with back arched with both hind and front legs tucked in under the body. The animal dropped down the following morning, showed no cornea reflex, and as it was in the last stage of the disease and struggled continually, it was killed on the seventy-fourth day of the experiment.

Horse 19650: Ingested approximately 42 kilograms of the dried stems, leaves, and seeds of the plant within seven weeks.

This animal received the dried plant in order to ascertain whether the inhalation of the dust played any part in the production of the lung lesions.

Result: The course of the disease resembled that described in horse 19662 to a very marked extent, so that a repetition of the symptoms seems unnecessary.

The animal was killed when *in extremis* on the sixty-first day of the experiment. The temperature which was elevated during the last week of the disease reached 104.8° F. before the animal was destroyed.

Post-mortem appearances and histology: The post-mortem appearances and histology of the affected organs of the above horses coincided with those cases described by Sir Arnold Theiler (1918).

Conclusion.—It has been definitely proved that *Crotalaria dura* " after prolonged storage is still capable of producing jaagsiekte in horses, a fact which is of considerable importance as far as the feeding of hay contaminated with this plant is concerned.

Erythrina caffra Thunb.

Common names: Kafferboom, coral-tree.

Origin: Onderstepoort, Pretoria.

The flowers were alleged to have caused death in chickens.

Rabbit: Received 60 grams of fresh flowers *per os*.

Result: Negative.

Neorautanenia coriacea C.A.Sm.

Origin: Newlands, Nylstroom, Transvaal.

The root of this plant is being used by the natives as a fish poison.

Goat 25123: Received 600 grams of the fresh root *per os*.

Result: Negative.

LILIACEAE.

Drimia purpurescens Jacq.

Common name: Jukbol.

Origin: P. L. le Roux, Pokkraal, Worcester.

Sheep 26056: Received 750 grams of fresh bulbs of the plant in the late seeding stage.

Result: Negative.

Ornithoglossum glaucum Salisb.

Common name: Cape Slangkop.

Origin: Mr. Freitag, Vlakfontein, Pretoria.

Sheep 27076: Received 650 grams of fresh leaves, flowers and unripe seeds of the above plant.

Result: The following morning the animal was found lying down. When it was approached, it jumped up and ran about the kraal in an excited manner. It was easily discernible that it had no perfect control over the muscles of the body, especially those of the hindquarters. It soon lay down on its side exhausted and struggling to rise. The pulse was imperceptible and the heart-beat very faint. There were pronounced cyanosis and clonic spasms of different groups of body muscles. The heart beat stopped while the respiration still continued for a few seconds. Death occurred within twenty hours after dosage.

Post-mortem appearances: Pronounced general cyanosis; on skinning, a large amount of blood flowed from the subcutaneous tissues; hyperaemia of the lungs; heart flabby and both ventricles markedly distended; oedema of the retropharyngeal mediastinal and bronchial lymph glands; degenerative changes in the liver with slight pigmentation; a pronounced acute duodenitis and jejunitis with numerous petechiae in the mucosa.

Ornithoglossum glaucum Salisb.

Common name: Cape Slangkop.

Origin: P. L. le Roux, Pokkraal, Worcester.

A limited amount of the plant was submitted for investigation.

Rabbit: Received 65 grams of fresh leaves, flowers and unripe seeds of the above plant on two consecutive days.

Result: Negative.

Discussion: MacOwen (1877), Professor of Experimental and Biological Science at Gill College, Somerset East, in giving evidence before the Cattle and Sheep Disease Commission in 1877, stated that "Cape Slangkop" had caused death in cattle. Hutcheon in quoting from one of Dixon's reports in 1894 says: "Since then I have had to treat sheep and goats in Griqualand West and Bechuanaland for the effects of eating Slangkop (*Ornithoglossum glaucum* Salisb.), and when attended to early I have found a mixture of linseed oil, a wineglassful, and a teaspoonful of powdered slaked lime an effective remedy."

As far as I could ascertain from the literature at my disposal no experimental proof of the toxicity of Cape Slangkop has as yet been brought forward.

MALVACEAE.

Sida rhombifolia L.

Common names: Pretoria bossie, taaiman.

Origin: C. de Nysschen, Rhebokfontein, P.O. Munnik, Pietersburg.

This plant was suspected of having caused "droukgalsiekte" in cattle.

Sheep 24692: Received 200 grams of dried leaves, stems and seeds of the above plant on two consecutive days.

Result: Negative.

Ochnaceae.

Ochna pulchra Hook.

Origin: Haakdoorndraai, Potgietersrust.

Sheep 28145: Received 3,600 grams of fresh young leaves and flowers in the course of seven days.

Result: On the fifth day of the experiment the animal suffered from an acute diarrhoea and tympanites was fairly severe. The pulse was accelerated and strong and the respiration hurried and more of the abdominal type.

The animal died on the sixth day of the experiment.

Post-mortem appearances: Green fluid from the nostrils and mouth; general cyanosis; pronounced hyperaemia of the lungs; ruminal contents in the bronchi, trachea, larynx and oesophagus; degeneration and pigmentation of the liver; degeneration of the myocard; a pronounced acute catarrhal duodenitis and jejunitis.

Polygonaceae.

Rumex nepalensis Spreng.

Origin: Roberts Heights, Pretoria.

The plant material was collected from a consignment of forage, which was suspected of having caused death in horses.

Sheep: 21161: Received on each of two consecutive days 300 grams of leaves, stems and seed of the plant in the late seeding stage.

Result: Negative.

Ranunculaceae.

Clematis brachiata Thunb.

Common names: Travellers' Joy, Oldman's Beard.

Origin: Somerset East.

Goat: 25123: Received 185 grams of the slightly wilted vines and flowers *per os*.

Result: Negative.

Two days later this animal received 750 grams of fresh vines and flowers of the plant growing at Onderstepoort with completely negative results.

Rubiaceae.

Gardenia thunbergii L. Fill.

Common names: Wilde katjiepiering, stompdoorn, bobbejaangif.

Origin: Rietfontein, Naboomspruit.

126 grams of driep ripe fruit = 84 grams exocarp and 42 grams endocarp.

Exocarp: 1 rabbit received 25 grams of dried exocarp.

Endocarp: 1 rabbit received 25 grams of dried endocarp.

Result: Negative.

Vangueria pygmaea Schltr.

Common name: Gousiekte bossie.

Origin: Pretoria District.

The plant, which was collected in the post-fruiting stage, had been stored at Onderstepoort for six years.

Sheep 24692: Received 400 grams of dried leaves daily except Sundays from 4.9.30.

Result: From 22.9.30 there were steady loss in condition and a progressive weakness. From 4.10.30 the animal, which had been of a particularly lively nature, was lying down frequently and was quickly exhausted when driven. These symptoms increased in severity until death took place during the night of 7.10.30.

The total amount of plant received amounted to 12 kilograms.

Post-mortem appearances: Pronounced general cyanosis; marked dilatation of both ventricles of the heart; consistence of the myocard was firmer to the touch than normal; but no appreciable change in the thickness of the walls of the ventricles.

Histology: The myocard did not show the typical gousiekte lesions.

SOLANACEAE.

Datura stramonium L.

Common names: Pietjie Laporte, stinkblaar, thorn-apple.

Origin: Onderstepoort.

One rabbit: Received 100 grams of the ripe seeds.

Result: Negative.

One rabbit: Received 100 grams of the fresh green leaves of the plant in the late seeding stage.

Each of the above animals received two 50-gram doses administered within an hour of each other.

Sheep 28412: Received 500 grams of the ripe seeds on each of two consecutive days.

Result: Negative.

Datura tatula L.

Common name: Blou stinkblaar.

Origin: Onderstepoort.

Sheep 25638: Received 750 grams of fresh leaves of the plant in the late flowering stage on each of two consecutive days.

Result: Negative.

Sheep 28266: Received 500 grams of the ripe seeds on each of two consecutive days.

Result: Negative.

Datura stramonium—Datura tatula Hybrid.

Quite a number of Datura specimens, which bore the characteristics of both *Datura stramonium* and *Datura tatula* were found growing mixed with the two latter plants. Ripe seeds were collected from these hybrid specimens and tested.

Sheep 28233: Received 500 grams of ripe seeds on each of two consecutive days.

Result: Negative.

I might mention here that all three of the above varieties of *Datura* were subsequently grown from the seeds of this hybrid.

Solanum incanum Linn.

Origin: Onderstepoort.

Goat 25123: Received 1,000 grams of fresh ripe fruits on each of two consecutive days.

Result: Negative.

Solanum tuberosum Linn.

Common name: Potato.

Origin: Onderstepoort.

One Rabbit: Received 45 grams of the fresh unripe fruits.

Result: Negative.

ZYGOPHYLLACEAE.

Zygophyllum foetidum Shr.

Origin: Mr. P. W. F. Weyer, P.O. Toekomst, Cape.

One Rabbit: Received 36 grams of fresh leaves, flowers and buds.

Result: Negative.

One Rabbit: Received 120 grams of fresh leaves, flowers and buds.

Result: Negative.

CONCLUSIONS.

1. 150 grams of *Mesembrianthemum anatomicum* Harv. (kougoed), a few drops of which is supposed to have a sedative effect on children, had no ill-effects on a rabbit.

2. 50 grams of specimens of *Psilocaulon* sp. (Nat. Herb. No. 8819), collected in the Willowmore district, caused death in rabbits, whereas 120 grams of these specimens tested two months after transplantation on the rockery in the poisonous plant garden at Onderstepoort, failed to produce any ill-effects in rabbits.

3. It was found impossible to kill sheep by drenching them with large amounts of the fresh green leaves and ripe seeds of *Datura stramonium*, *Datura tatula* and of the hybrids of these two plants.

4. The following plants, of which no toxic records could be found in the available literature, have been definitely proved toxic.

(*a*) *Psilocaulon* sp. (Nat. Herb. 8819).
(*b*) *Haemanthus amarylloides* Jacq.
(*c*) *Narcissus jonquilla* Linn.
(*d*) *Nerine lucida* Herb.
(*e*) *Asclepias fruticosa* L.
(*f*) *Aster filifolius* Vent.
(*g*) *Gerigeria pectidea* Harv.
(*h*) *Senecio isatideus* D.C.
(*i*) *Ornithoglossum glaucum* Salisb.

The last mentioned plant has been suspected but never before proved toxic experimentally.

5. *Crotalaria dura* and *Vangueria pygmaea* were found not to have lost their toxicity after five and six years' storage respectively.

ACKNOWLEDGMENTS.

I wish to express my indebtedness to Dr. de Kock, Head of the Department of Pathology, for valuable suggestions made by him, and to Dr. Phillips, Principal Botanist of the Division of Plant Industry, Pretoria, for the favour of identifying plants, for reading through this article, and for valuable information supplied by him.

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Investigations into the Cause of Alopecia (Kaalsiekte) in Kids and Lambs.

By D. G. STEYN, B.Sc., Dr.Med.Vet., Veterinary Research Officer, Onderstepoort.

INTRODUCTION.

"KAALSIEKTE" (Alopecia), which literally means "naked-disease," is the term applied to a disease in lambs of cross-breeds of sheep and in kids, the most outstanding symptom of which is partial or complete loss of the coat. Hence the appropriate name assigned by farmers to this disease.

Alopecia, although it had existed in the Willowmore, Uniondale and other neighbouring districts for the last seventy or eighty years, according to reliable information supplied by elderly and experienced farmers, was for the first time investigated and reported upon by Van Rensburg (1925), the then lecturer in veterinary science at the Grootfontein School of Agriculture, Middelburg, Cape Province. Specimens of the skin and thyroid glands of affected kids were forwarded to Onderstepoort for histological examination. The skin showed a crustaceous eczema and the thyroids revealed no specific changes.

As the affected area in the Willowmore and Uniondale districts alone carries approximately two hundred and fifty thousand Angora and mixed breed of goats, it was decided to have this disease investigated.

INCIDENCE AND STATISTICS.

The disease is said to be limited to "sour veld." Mr. Botha, who has had many years of experience as sheep inspector in the areas where alopecia is prevalent, has informed me that he has also seen the disease in the Cradock, Hofmeyr, Tarkastad, Middelburg (Cape), Colesburg, Graaff-Reinet, Pearston and Jansenville districts. In these districts, he continued, the disease invariably appears on farms with deficient soil which produces very little, if any, edible vegetation of a high nutritive value. In the course of my investigations I had ample opportunity of verifying the latter statement.

Mr. De Villiers, Government Veterinary Officer, Middelburg, Cape, who investigated this disease in the Cradock district, reported that it is most prevalent in the mountainous parts of that district. This has been my experience in the Willowmore and Uniondale districts, where the disease is limited to the north-western part of Uniondale and the south-eastern part of Willowmore.

It is impossible to express in exact figures the losses caused by this disease. These are, however, enormous, as can be guaged by the fact that in the Willowmore and Uniondale districts alone over two hundred thousand susceptible small stock are running in the affected areas. On many a farm it is impossible to rear a single kid or lamb of mixed breeds of sheep. On such farms it is a generalf practice not to rear any kids or lambs of cross-breeds of sheep, but to buy three to six months old kids and lambs, which then mature on these farms without any apparent ill-effects.

In addition to the losses amongst the young of the small stock the owner of an "Alopecia farm" suffers a direct heavy financial loss due to the fact that such a farm loses considerably in value as far as the grazing capacity for small stock is concerned.

Time of the Year.

The severity of the disease varies considerably from year to year. In years when late winter or early summer rains fall the disease is much more prevalent than in dry years, during which the disease either does not appear or occurs in a very mild form only, even on the most notorious Alopecia farms. The disease is most prevalent during the period August to October, although severe outbreaks have been known to occur during May, June, and July. Personally I have seen the disease in June, 1930, in the Willowmore district.

Species of Animals Affected.

Cases have appeared in the lambs of crossbreeds of all sheep, in Angora kids and in the kids of crossbreeds of goats. The disease exhibits itself in from four to fourteen days old lambs and kids and rarely makes its appearance in these animals after the age of two weeks. During the worst outbreaks cases have been reported to have occurred in kids up to one month old. The affected areas carry the above breeds of small stock only and no Merino sheep. None of the many farmers interviewed could give me any information regarding the occurrence of the disease in Merino lambs.* Up to the present no cases in Merino lambs have been reported. Donkeys, horses and cattle also run in the alopecia areas, but no cases of this disease have ever been known to appear in the young of these animals.

Morbidity and Mortality.

The morbidity depends to a considerable extent on the rainfall. It is highest after late winter or early summer rains have fallen, when the morbidity might be as high as 100 per cent. We must bear in mind the fact that the kidding season in the affected areas extends usually from the beginning of August to the end of October.

The mortality depends largely on the care given to the affected animals. In cases of negligence the mortality might be as high as 90 per cent., whereas it could be reduced to 50 per cent. or less with proper treatment. This point will be discussed under "Treatment."

* Van Rensburg (1925), however, mentions some farmers to have reported that they have seen Alopecia in Merino lambs.

Symptomatology.

The symptoms can best de divided into primary and secondary.

Primary Symptoms.

These are the shedding of the coat and diarrhoea. The former symptom oppears in about 95 per cent. of the cases and frequently is associated with diarrhoea. It rarely happens that the affected kids and lambs develop a pronounced diarrhoea and die before loss of hair occurs.

On careful observation the first discernible symptom is itching and the affected animals can be seen scratching and biting their sides. At this early stage the hair, especially over the sides, can be easily removed by hand. Macroscopically the skin on the affected parts of the body appears quite normal. In a bad case such an animal loses practically its whole coat overnight and it continually seeks shelter. In mild cases the coat is shed over a period of a few days provided the affected animal survives the diarrhoea.

Invariably the first bald patches are to be seen over the shoulder blades and the upper half of the hind legs. (See Fig. V.) By pulling the hair on these parts an affected case can be detected in its earliest stages. Many an early case can be picked out by the peculiar ruffled appearance of the hair over these parts.

As soon as diarrhoea sets in the animals show inapptence, listlessness and, frequently, fever. In cases of severe diarrhoea the animals die within one to three days without loss of hair.

In the course of a few hours to a few days, depending on the severity of the case, the affected kids and lambs become completely bald (see Fig. II), with a little hair left on the lower parts of the legs, the tips of the ears and tail, and on the head. These animals present a most peculiar appearance. In this state the animals show symptoms of a marked general disturbance, e.g. fever, diarrhoea, inappetence, marked depression, staggering gait and, ultimately, inability to rise.

The hair on the coloured patches of the skin (in mixed breeds of sheep and goats) is much more resistant to the effects of the toxin than that on the unpigmented parts, with the result that the coloured patches are still covered by hair, whereas the unpigmented parts of the skin are completely bald. Likewise, black and brown animals are more resistant to Alopecia. However, in bad cases these animals also lose their hair, provided they do not die within a short time from diarrhoea.

In addition, the effected kids develop an acute conjunctivitis, keratitis and rhinitis (see Fig. VI). Permanent loss of eyesight is, however, very rare.

Secondary Symptoms.

These are an acute dermatitis, a pronounced diarrhoea, an obstruction of the gastro-intestinal tract by hairballs, and an acute catarrhal pneumona.

The acute dermatitis is caused most prabably by the action of sunlight on the unprotected skin (see Fig. III). It commences on the hairless patches with an intense reddening, swelling, painfulness

and, later on, exudation, and, in the course of time, hard crusts are formed on the skin. It is evident from Fig. III that such animals experience severe pain when walking or standing with straightened legs and back, as this causes stretching of the skin.

A diarrhoea almost invariably accompanies the process of shedding the hair. The diarrhoea in Alopecia in kids and lambs may be caused either primarily by the toxin, as it is present in cases where no loss of hair occurs, or secondarily by the irritation produced by the ingestion of large amounts of shedded hair, or by both these factors. Affected kids can be seen pulling out mouthfuls of hair from the itching skin, chewing these and swallowing them.

Rarely, kids can be seen to shed the coat and die within a comparatively short time without developing a diarrhoea. In these cases the post-mortem reveals one or more hairballs completely obstructing the pyloric portion of the abomasum (see Fig. IX). On post-mortem the abomasum is distended by intense malodorous gases and its contents are in a state of advanced decomposition. Many farmers have informed me that they have lost kids from the effects of hairballs up to three months after these animals had recovered from Alopecia.

In cold weather a large percentage of the bald kids develop a fatal acute catarrhal pneumonia if the necessary provisions for shelter are not made.

Post-mortem Appearances.

As far as the external lesions are concerned these coincide with those described under Primary and Secondary Symptoms. An acute catarrhal gastro-enteritis is present in cases which have exhibited a diarrhoea. In all cases where Alopecia was present the post-mortem reveals an enormous amount of hair mixed with the gastro-intestinal contents and hairballs varying in size. As has been described before, the latter might cause death before the gastro-intestinal irritation has been produced.

An acute catarrhal pneumonia frequently is the cause of death in hairless kids and lambs and may be complicated by one or more of the other secondary conditions.

Nature and Cause of the Disease.

This aspect of the disease will be discussed under "Experiments to determine the Cause of Alopecia in Kids and Lambs."

Treatment.

The careful nursing of the affected animals is of much greater importance than the treatment which is not of much use. Treatment can be carried out on general principles.

Mild oily laxatives, for example, raw linseed oil, are indicated to remove the hair and hairballs from the gastro-intestinal tract. This treatment must be commenced as soon as the kids are noticed to bite and scratch, as in the course of a day or two the hairballs reach such dimensions as will render their escape from the abomasum into the small intestine impossible.

In diarrhoea small quantities of raw linseed oil and limewater will prove beneficial.

In pneumonia treatment generally is of no avail, but such could be carried out on general principles.

The dermatitis can be treated with liniments to prevent the skin from hardening and to allay the irritation, and farmers have reported very favourably on the effects of lanolin on the bald and burnt skin.

Prevention.

Prevention as far as the primary symptoms are concerned will be discussed in the second part of this paper.

Many a farmer, although experiencing a high percentage of Alopecia amongst his animals, has reduced his losses to a minimum by erecting a small shed at the kraal where the kids and lambs are kept. These sheds afford the necessary shelter against cold and the burning rays of the sun, with the result that pneumonia and dermatitis rarely develop.

EXPERIMENTS TO DETERMINE THE CAUSE OF ALOPECIA IN KIDS AND LAMBS.

A. Experiments on the Farm Skilpadbeen, Willowmore.

Preliminary Investigations.

The author has investigated the disease on a number of farms in those parts of the Uniondale and Willowmore districts where the disease is most prevalent. In addition, Alopecia-free farms were visited for purposes of comparison.

First of all the nature of the disease had to be settled. Was it a deficiency disease or infectious disease or a plant intoxication?

The following facts gathered in the course of the investigation pointed strongly in favour of plant poisoning:—

(*a*) The disease does not appear when the pregnant goats and cross-breeds of sheep are turned on to green barley or oat-lands fourteen days before kidding and lambing commences and kept there until fourteen days after kidding and lambing. The same applies when the pregnant goats and sheep are allowed to graze high up in the mountains for the above period. The disease is also known to be more severe on certain parts of one and the same farm than on other parts.

(*b*) The disease is most prevalent in years when the rainfall is at its highest. At such times there is a luxuriant growth of herbage and we must consider that certain plants might be eaten only when in the flowering stage. In addition,

in good years the milk yield is much higher than in years of drought. Farmers have informed me that in years of drought the morbidity may be 1 per cent. or even less, whereas on the same farm the morbidity might be as hi as 100 per cent. during years with a high rainfall.

(*c*) The disease can be controlled to a certain extent by partially emptying the mother's udder before the young ones suck. This method of preventing the disease is practised by many a farmer with quite a fair amount of success. The less milk the kids get, the less "poison" they ingest.

(*d*) Twins and triplets are very much less susceptible to the disease than in cases where only one kid or lamb sucks the mother. This again is a case of the young receiving less milk and consequently less "poison."

(*e*) No more new outbreaks are experienced as soon as the kids and lambs are weaned.

(*f*) Kids and lambs are never born bald, the earliest cases of Alopecia appearing in three days- old kids and lambs. It is most prevalent in kids and lambs from four to fourteen days old, less prevalent in three weeks old kids and lambs and is rarely seen after these animals have passed the age of three weeks.

(*g*) It is a common experience that kids or lambs sucking a mother-goat or sheep, whose young has died from the effects of Alopecia, will also contract the disease.

It has been suggested that Alopecia is due to a deficiency of iodine. However, the above-mentioned points, as well as the fact that the kids and lambs are born in a state of perfect health with a perfectly normal coat, speak against this theory. In the face of the above facts it is unnecessary to elaborate on the symptoms of iodine deficiency.

It is of interest to mention the views expressed by the farmers as to the cause of the disease. These can be summed up as follows:—

(*a*) A number of farmers ascribed the disease to the mother-goats and sheep eating the so-called "opslag" after the rains. The term "opslag," as used by the farmer, may include any plant whose growth is dependent on the late winter or early summer rains.

(*b*) The following plants have been incriminated: The geelbos (*Lopholaena Raultii*); the beesbossie, also known as the bitterkarroo, brandbossie; bitterbossie (*Chrysocoma tenuifolia*); Euphorbia species; and a number of other plants. Some farmers maintained that the mothers had to ingest more than one plant at the same time in order to cause the development of Alopecia in their young.

(*c*) Many believed the water to be the source of the trouble.

(*d*) Others again were baffled by the disease and could express no opinio nas to its possible cause.

(*e*) Many a farmer regarded Alopecia as a deficiency disease and claimed good results by supplying the mothers with licks containing iron sulphate and bonemeal.

(*f*) Some farmers maintain that they combat the disease very successfully by feeding a salt-bonemeal lick to the breeding stock.

The results of my investigations force me to conclude that Alopecia in kids and lambs is a case of definite plant poisoning, the pathogenesis of which is as follows: The mother of the affected kids or lambs ingests a plant, which apparently causes no harmful effects in the full-grown animals, but the toxin of which is secreted in the milk and in this way produces the so-called Alopecia in the kids and lambs.

The water as a possible source of the trouble could be eliminated, as the disease could be prevented by allowing the mothers to graze on green oats or barley lands, whereas the water supply remained the same.

A careful botanical survey of a number of affected and unaffected farms was then made. The unaffected farms and the "Alopecia-free" and "Alopecia" portions of the affecte dfarms were carefully examined with the result that the number of suspicious plants were reduced to two, namely, the beesbossie (*Chrysocoma tenuifolia*) and the Bothablom (*Polygala teretifolia*).

It was then decided that experiments should be conducted with these two plants.

Arrangements were accordingly made with Mr. M. J. Ferreira, Skildpadbeen, Willowmore, to conduct the experiments on his farm, which in the past has been so bad with the disease that the owner had practically stopped breeding kids and lambs, and resorted to the method of buying three to six months' old kids and lambs for purposes of speculation.

B. Field Experiments in the Willowmore Destrict.

The undermentioned experiments with highly pregnant Angora goats were commenced on the 12/8/30, approximately two weeks before kidding was due.

EXPERIMENT I.

To determine whether *Chrysocoma tenuifolia* Berg. is the cause of Alopecia in kids.

Common names of plants: Bitterkarroo, beeskarroo, bitterbossie, brandbossie.

Fifteen goats were fed as follows:—

(*a*) Bitterkarroo only: 5 goats.

(*b*) Bitterkarroo + 0.1 gram potassium iodide per head daily: 5 goats.

(*c*) Bitterkarroo + two tablespoons of salt-bonemeal lick (1:2) daily: 5 goats.

These fifteen animals were placed in a specially constructed wire-netting kraal twenty-six by sixteen yards, from which all the vegetation, except the bitterkarroo, was removed. The animals immediately commenced eating the bush and completely cleared the kraal within six hours. From the 13/8/30 the animals received approximately sixty pounds of the freshly cut bush per day. The flowering plant only was utilized in the feeding experiments and was collected by cutting off the upper four inches of the bush. The material, which was scattered in a shady place, in order to prevent rapid wilting, was very eagerly eaten by the goats.

All the experimental goats received their drinking water from the same source as the flock in order to ascertain whether the drinking water played any part in the causation of the disease.

The five goats in group (*b*) were dosed daily with 0.1 gram potassium iodide in order to exclude an iodine deficiency. The potassium iodide was dissolved in ordinary spring water and dosed by means of a syringe.

Each of the five goats in group (*c*) received daily two tablespoonfuls of a mixture of one part of salt and two parts of bonemeal, in order to determine whether these substances had any direct effect on the disease, preventively or curatively. I might mention here that many a farmer in the "krimpsiekte" areas of the Uniondale and Willowmore districts, where this disease is caused by *Cotyledon ventricosa* and *Cotyledon wallichii*, has informed me that they have reduced their losses from "krimpsiekte" to a negligible number by allowing their stock free access to a salt-bonemeal lick.

It was the intention to include an additional experiment by daily force-feeding twenty pregnant goats with the salt-bonemeal lick and allowing them to run with the flock, which had to serve as controls. This experiment was abandoned, as Mr. Ferreira allows all his animals free access to a salt-bonemeal lick.

On the fourth day after the commencement of the experiment, that is, after each animal had ingested approximately sixteen pounds of the flowering tops of the freshly cut plant, all the animals showed a fairly marked diarrhoea, which in the course of a few days became very acute. The animals exhibited pronounced straining, evacuating varying quantities of a very fluid greenish material mixed with large amounts of mucus which in a few animals was mixed with blood. There was extremely rapid loss in condition and the animals consumed enormous amounts of water. The eyes were sunken and the nostrils showed a dirty mucous discharge.

The visible mucous membranes were extremely pale and there was complete absence of appetite. Furthermore, the animals exhibited a general weakness, which progressed until they were unable to rise. The respiration was very rapid, costo-abdominal and shallow. The pulse was accelerated and in the course of time became very irregular and ultimately imperceptible. In the last stage of the disease the animals lay on their sides utterly exhausted and helpless until death supervened.

Four of the fifteen animals aborted in the course of the disease, and it is of interest to note that three of these animals were receiving 0.1 gram potassium iodide daily.

Three animals died and one was killed *in extremis* within sixteen days after commencement of the experiment.

Post-mortem Appearances.

Anaemia, cachexia, hyperaemia of the lungs and liver; heart extremely flabby and with both ventricles markedly distended with coagulated blood; acute catarrhal gastro-enteritis. The four animals showed advanced pregnancy.

All the affected animals were treated with a mixture of limewater and raw linseed oil with very unsatisfactory results. As mentioned above, four died and the rest recovered extremely slowly.

Only seven pregnant animals were left and of these one had to be discharged on account of its bad condition. Another nine available pregnant Angora goats were placed in the experiment in order to bring the number up to fifteen and the experiment continued from the 1/9/30.

During field observations it was noticed that both sheep and goats fed extensively on the flowering bitterkarroo without any ill-effects. It was thought that symptoms of bitterkarroo poisoning could be prevented when additional food were given to the experimental animals. Accordingly from 1/9/30 the fifteen experimental animals received approximately sixty pounds of the freshly cut flowering bitterkarroo in the morning and as soon as this had been ingested they were offered twenty pounds of lucerne hay and forty pounds of green barley. Of the lucerne hay and barley they took variable quantities, whereas of the bitterkarroo each animal daily took approximately four pounds of the freshly collected flowering plant. In spite of the fact that the animals were daily fed as above from the 1/9/30 to the 27/9/30, they developed no symptoms of ill-health.

Eleven out of thirteen kids born from the above goats developed typical symptoms of Alopecia as it occurs under natural conditions. Of the affected kids eight died and one was killed *in extremis*.

The results of this experiment are summarised in the following table.

TABLE I.

Chrysocoma tenuifolia *fed to Pregnant Goats.*

No. of Pregnant Goat	Material Received.	Date of Birth of Kids.	Date on which Symptoms of Alopecia Appeared in Kids.	Interval between Date of Birth and Date of Appearance of Symptoms.	Remarks.
1	Bitterkaroo only	—	—	—	Died on 27/8/30, from effects of Bitterkaroo.
2		—	—	—	Died on 26/8/30, from effects of Bitterkaroo.
3		Aborted on 27/8/30	—	—	Discharged.
4		—	—	—	Killed *in extremis* on 28/8/30. Condition due to ingestion of Bitterkaroo.
5		9/9/30	18/9/30	9 days	Alopecia diarrhoea. Died on 1/10/30.
6		11/9/30	14/9/30	3 days	Slight alopecia. No diarrhoea. Died on 14/9/30.
7		16/9/30	23/9/30	7 days	Alopecia and diarrhoea. Died on 1/10/30.
8		16/9/30	27/9/30	11 days	Alopecia and diarrhoea. Died on 29/9/30.
9		19/9/30	24/9/30	5 days	Alopecia and diarrhoea. Died on 30/9/30.
1	Bitterkaroo + Potassium Iodide	Aborted on 19/8/30	—	—	Discharged.
2		,, 20/8/30	—	—	Discharged.
3		,, 23/8/30	—	—	Discharged.
4		7/9/30	—	—	No alopecia developed.
5		9/9/30	—	—	Mother had no milk, hence discharged.
6		16/9/30	22/9/30	6 days	Alopecia + diarrhoea. Died on 26/9/30.
7		Aborted on 16/9/30	—	—	Discharged.
8		Aborted on 16/9/30	—	—	Discharged.
1	Bitterkaroo + Salt-bonemeal Lick	18/8/30	—	—	Kid died on 22/8/30. Cause of death unknown. Mother discharged.
2		—	—	—	Died on 27/8/30 from effects of Bitterkaroo.
3		8/9/30	18/9/30	10 days	Alopecia + diarrhoea. Died on 30/9/30.
4		12/9/30	19/9/30	7 days	Alopecia. Died on 26/9/30.
5		16/9/30, Twins	27/9/30	11 days	One kid developed alopecia and recovered, whereas the other one remained healthy.
6		19/9/30	23/9/30	4 days	Alopecia and diarrhoea. Killed *in extremis* on 27/9/30.
7		19/9/30	27/9/30	8 days	Alopecia + diarrhoea. Recovered.

Discussion.

From the above table it is evident that—

(*a*) five goats of the potassium iodide group aborted, and one of the bitterkarroo group;

(*b*) eleven out of thirteen experimental kids developed typical symptoms of Alopecia, and of these eight died and one was killed *in extremis;*

(*c*) only one of the twin kids developed Alopecia;

(*d*) the earliest case of Alopecia appeared three days after birth and the latest one eleven days after birth;

(*e*) death occurred within two to twelve days after the appearance of symptoms. One kid died on the day Alopecia was noticed;

(*f*) three pregnant goats died from the effects of the bitterkarroo and one was killed *in extremis.*

The appearance, course, symptoms and post-mortem appearances of Alopecia as it was noticed in the experimental kids, coincided completely with those encountered in the natural cases of the disease.

The mother-goats, although well-fed, were much worse in condition and consumed much larger quantities of water than the fifteen animals in Experiment II, which, in addition to the "Bothablombos," received the same amount of lucerne hay and green barley as the goats in Experiment I.

EXPERIMENT II.

Polygala tercti folia, Thunb.

Common name: Bothablombos.

Fifteen pregnant Angora goats were placed in a pen erected about fifty yards away from that of Experiment I and received daily sixty pounds of the fresh flowering tops of the "Bothablombos" from the 12/8/30 to the 27/9/30. The animals picked off all the flowers and took very little of the leaves of the plant, with the result that they ingested only about thrity of the sixty pounds of plant offered to them. As the animals steadily lost in condition they received twenty pounds of lucerne hay and forty pounds of green barley in the afternoon.

(*a*) Bothablombos only: 5 goats.

(*b*) Bothablombos + 0.1 gram potassium iodide per head daily: 5 goats.

(*c*) Bothablombos + two tablespoonfuls of salt-bonemeal lick (1:2) per head daily: 5 goats.

Up to the 1/9/30 two goats in (*b*), one in (*c*), and one in (*a*) aborted. These four animals were replaced by four pregnant goats on 1/9/30. On 5/9/30 another potassium iodide goat aborted. The rest of the goats gave birth to normal kids. At the time the experiment was discontinued there were ten kids ranging in age from two to five weeks. Both the kids and their mothers were in good condition and perfect health.

Discussion.

The fresh "Bothablombos" in the quantities fed had no deleterious effects on the experimental animals. Three potassium iodide goats, one salt-bonemeal goat and one "Bothalombos" goat aborted.

EXPERIMENT III.

Twenty pregnant Angora goats, which daily received 0.1 gram potassium iodide from 12/8/30, were allowed to run with the flock. As a high percentage of abortions occurred in the animals receiving the potassium iodide, the quantity was reduced to 0.05 gram daily from 1/9/30. From this date onwards all the experimental animals in the potassium iodide groups received 0.05 grams per head.

Up to 14/9/30 five of the twenty goats aborted. At the time the experiment was discontinued the fifteen remaining goats had kids ranging in age from eleven days to six weeks. Amongst these kids only one very light case of Alopecia occurred.

EXPERIMENT IV.

Twenty pregnant Angora goats, each of which received daily 5 grams of sulphur, were allowed to run with the remainder of the flock.

On the 27/9/30, when the experiment was discontinued, the kids ranged in age from twelve days to six weeks. Only one case of Alopecia appeared amongst these kids. It is of interest to note that no abortions occurred in this group of animals.

CONTROLS.

The rest of the flock of pregnant goats, numbering about three hundred, which had free access to a salt-bonemeal lick (1:2), was kept as controls.

As I had advised the owner to keep the pregnant animals away from the "bitterkarroo" veld, the flock of pregnant goats were allowed to graze high up in the mountains during daytime, and only had the apportunity of feeding on the "bitterkarroo" in the evening when they were brought home and early in the morning when they were driven out.

This accounts for the extremely low percentage, namely, 2.4 per cent., of Alopecia which occurred in the flock, of which two hundred and fifty had already kidded at the time the experiment was discontinued. Of these animals six had aborted and amongst the kids only six cases of Alopecia appeared.

The owner informed me that in good years, such as 1930, up to 90 per cent. of his kids developed Alopecia.

Cotyledon wallichii and *Cotyledon ventricosa* must be considered as a possible cause of the abortions in the control flock, as these two plants, especially the latter, grow luxuriantly on the mountain slopes. Personally, I saw quite a number of cases of "krimpsiekte" in the control flock, and one goat had actually aborted twins during an attack of "krimpsiekte."

Experiments Conducted at Onderstepoort.

Since it was established that *Chrysocoma tenuifolia* (bitterkarroo) is the cause of Alopecia, which occurs so extensively in kids and lambs of mixed breeds in the Willowmore and neighbouring districts, it was decided to continue the investigations at Onderstepoort. The first point that had to be settled was whether the plant in the dry state was capable of producing the disease. This naturallly was of the utmost importance, as it was the most important factor to determine whether or not it would be possible to conduct experiments at Onderstepoort. As will be seen in the course of the undermentioned experiments, the dried plant was found to produce the disease, and it was proposed to investigate the following points in connection with Alopecia.

(*a*) The susceptibility of Merino lambs to Alopecia.

(*b*) The age factor. This is a point of enormous practical and economical importance. How long before kidding must the pregnant goats and sheep be removed from the "bitterkarroo" veld in order to prevent Alopecia? Experienced farmers maintain that no cases of Alopecia will appear if the pregnant animals are removed from "Alopecia veld" fourteen days before kidding and kept away from such veld until the kids are fourteen days old. This "time limit" could be easily determined by experiment. It will be realized that this "time limit" is of the utmost importance, as the feeding of thousands of pregnant animals involves large sums of money and frequently is impossible. It is, therefore, in the interest of the farmers concerned to know exactly how long to keep their animals away from "Alopecia veld."

(*c*) The susceptibility of other species of animals to Alopecia.

(*d*) Will the young of all species of animals shed their coat when taking the milk of an "Alopecia goat or sheep"?

(*e*) Must the mother goat or sheep prepare the Alopecia-toxin in its system or is it contained as such in the plant?

(*f*) Will the "bitterkarroo" from "Alopecia-free" areas also produce the disease? It is an interesting fact that Alopecia occurs in certain parts of the affected districts only. In the Willowmore and Uniondale districts the disease is very prevalent in the mountainous parts, whereas it is of extremely rare occurrence on the even veld. The grazing on the "Alopecia farms" is very poor, with the result that the animals have to rely to a large extent on the "bitterkarroo" for their food, whereas the veld of the "Alopecia-free" farms is of a much superior quality. Is the quality of the veld or a difference in the toxicity of the responsible plant in different localities the determining factor in the occurrence of Alopecia?

(*g*) The isolation of the "Alopecia-toxin."

(*h*) The cause of the shedding of the coat and the nature of the action of the toxin on the skin.

(*i*) Will the plant in all its stages of development produce Alopecia.

In the course of time an attempt will be made to elucidate all the above points.

Preliminary Experiments.

EXPERIMENT I.

To ascertain whether the plant in the dry state will still produce Alopecia?

Highly pregnant Angora goats obtained from the Willowmore district were employed in these experiments. The "Bitterkarroo" bush was collected in the flowering stage on the farm Skildpadbeen, sun-dried and forwarded to Onderstepoort.

Goats 29207 and 29214 were starved for twenty-four hours and then offered the dried "bitterkarroo." As nothing was ingested during the following twenty-four hours, the dried bush was cut up coarsely and mixed with lucerne hay. During the following three days the animals did not touch the mixture. As a good milk-yield is essential for the production of Alopecia it was thought inadvisable to continue starvation, and drenching was resorted to in all the following experiments.

The above animals received daily (except Sundays) 400 grams of the dry plant from the 27/10/30. On the 8/11/30 No. 29207 aborted and was discharged. As the abortion was most probably caused by the "bitterkarroo," this was an indication that the animals were receiving too large quantities of the plant. Consequently goat 29214, together with goats 29206 and 29203, the latter two having been added to the experiment on the 10/11/30, received daily (except Sundays) 200 grams of the plant until the date of lambing, when the dose was increased to 400 and 800 grams on alternate days.

Result.

Goat 29203.—This animal kidded on 30/11/30 (Kid No. 29371). On 16/12/30 it accidently inspired some of the drenching material with fatal results. Up to the time of death this animal had received 12.4 kilograms of the dried plant in the course of five weeks without any deleterious effects.

Kid 29371 (*born on* 30/11/30).

9/12/30—Diarrhoea.

10/12/30—Diarrhoea with straining. At 2 p.m. the animal was noticed stamping with the hindlegs, swishing the tail, running about and biting at its sides and hindlegs. It was seen chewing and swallowing the hair which had been pulled out during the biting. On closer examination it was found that the hair over the shoulder-blade, the sides and the lateral aspect of the hind legs could be removed very easily (see Fig. V). Temperature 103.4° F. The animals sought shade keenly. Macroscopically the skin appeared perfectly normal.

11/12/30—Diarrhoea. Sides almost completely hairless; hair on neck and back easily removable. Temperature 103.8° F. Pronounced acute catarrhal conjunctivitis. Losing in condition. Pulse 120, strong. Inappetence.

12/12/30—Diarrhoea pronounced. Hairless parts of the skin on the sides are red, warm, painful and swollen. Temperature 103.6° F. Pulse 124, strong. Inappetence.

13/12/30—Diarrhoea pronounced. Losing in condition. Shedding of the coat continued. Acute catarrhal conjunctivitis. The hairless portion of the skin shows an acute dermatitis. Animal walking with stiff legs, as movement causes stretching of the inflamed skin with consequent pain. Temperature 104.4° F. Animal shows symptoms of severe irritation of the skin. Pulse 118, strong. Inappetence.

14/12/30
15/12/30
16/12/30 } Condition as on 13/12/30. As the mother goat 29203 died, on 16/12/30, the kid was hand-reared from this date onwards with cow's milk. Inappetence.

17/12/30—Diarrhoea pronounced. Losing in condition and very apathetic. Moist dermatitis. Still shedding coat. A bilateral purulent conjunctivitis with the lids of the eyes glued together. An increased discharge from the nostrils. Temperature 103.6° F. The inflamed skin over the lateral aspect of the thigh shows a dry crustaceous dermatitis with deep blood-stained cracks (see Fig. VII). Pulse 132, strong. Inappetence.

18/12/30—Diarrhoea pronounced. Condition bad. Hairless portion of skin swollen, reddened, painful and hard. Animal appears hidebound, stands with back arched and moves with great pain and difficulty. Temperature 103.4° F. Pulse 128, strong. Inappetence.

19/12/30
20/12/30 } Condition as on 18/12/30. Animal almost hairless. Little hair left on the legs, back and head. Diffuse crustaceous dermatitis. Inappetence.

21/12/30—As on 20/12/30.

22/12/30—New hair appearing on the first hairless patches. Diarrhoea pronounced, acute purulent conjunctivitis and rhinitis. Diffuse crustaceous dermatitis, with bleeding cracks. Temperature 102.8° F. Not feeding. Condition very bad. Pulse 124, strong.

The above described skin lesions and symptoms culminated in death during the night of 26/12/30.

Post-mortem Appearances.—Almost complete hairlessness with new coat appearing on some parts of the skin; cachexia; anaemia; acute purulent conjunctivitis and rhinitis; acute crustaceous dermatitis; hyperaemia of the lungs and liver; marked atrophy of the spleen; hairballs in rumen [see Fig. IX (*a*)] and abomasum [see Fig. IX (*b*)], the latter completely obstructing the pyloric portion of the abomassum; acute catarrhal duodenitis, jejunitis and colitis; entire gastro-intestinal tract completely devoid of ingesta.

Goat 29214.—This animal gave birth to a normal kid (No. 29373) on the 5/12/30. From 27/10/30 to the 5/12/30 goat 29214 received 23.2 kilograms of the dry plant without developing any symptoms of ill-health.

Kid 29373 (*born on* 5/12/30).

11/12/30—Stamping with hind feet, swishing the tail, running about, and biting at external surface of hind legs. On closer examination the hair on the sides and external surfaces of the legs and to a slighter extent on the back can be easily removed. Temperature 103.8° F. Pulse and respiration normal. No diarrhoea.

12/12/30—Hairless patches on the sides and back. Temperature 103.8° F. Animal exhibits symptoms of marked irritation of the skin. Biting at the sides, chewing and swallowing the extracted hair.

13/12/30—As on 12/12/30.

14/12/30—Sides and lateral aspect of the thighs completely hairless. The hairless portions of the skin show signs of inflammation.

15/12/30—Diarrhoea. Shedding of coat progressing. Temperature 103.4° F. Slight catarrhal conjunctivitis.

16/12/30—Pronounced diarrhoea. Losing in condition. Lots of hair coming off all over body. Acute catarrhal conjunctivitis and rhinitis. Temperature 103.4° F. Pulse 118, strong.

17/12/30 } Condition growing worse.
18/12/30 } Pronounced diarrhoea and loss in condition.
19/12/30 · Acute purulent conjunctivitis and rhinitis.
20/12/30 } Shedding of coat and dermatitis progressing. Tem-
21/12/30 } perature 102.8°. Pulse 124, strong.

22/12/30—Pronounced diarrhoea. New hair appearing on the hairless portion of the skin. From this date onwards there was steady improvement in the condition of the animal until complete recovery on 5/1/31.

Goat 29205.—This animal kidded on 20/11/30 (Kid No. 29368). From 10/11/30 up to the time of death from an unknown cause on 11/12/30 it had received 12.6 kilograms of the dry "bitterkarroo."

Goat 29205 was in perfect health up to the 11/12/30 and was found dead in the stable the next morning.

Post-mortem Appearances.—Heart in diastole with both ventricles markedly distended with coagulated blood; marked hyperaemia and slight oedema of the lungs; degenerative changes in the liver; at the junction of the intermediate zone and medulla of the kidney there was deposition of a gritty material; haemorrhages in the peritracheal tissues; a slight chronic duodenitis and jejunitis.

Histology.

Myocard—Slight hyperaemia.

Liver—Slight hyperaemia, severe fatty changes more marked at the peripheri.

Kidneys—Marked fatty changes; peculiar bodies in medulla of unknown significance.

Kid 29368.—This animal remained perfectly healthy in spite of the fact that it was suckled by its mother, which was drenched in the same way as goats 29203 and 29214 for three weeks.

TABLE II (Experiment I).

Dried Willowmore "Bitterkarroo."

Goat No.	Date on which Dosage Commenced.	Period of Dosage.	Quantity of Dry Plant Received.	Kid No.	Date of Birth.	Date of Appearance of Alopecia Symptoms.	Remarks.
29207	27/10/30	11 days.	4 Kg.	—	—	—	Aborted on 8/11/30.
29203	10/11/30	35 ,,	12·4 ,,	29371	30/11/30	9/12/30	Diarrhoea appeared on 9/12/30, and Alopecia on 10/12/30. Kid died on 26/12/30.
29214	27/10/30	29 ,,	23·2 ,,	29373	5/12/30	11/12/30	Alopecia. Complete recovery had taken place on 5/1/31.
29205	10/11/30	33 ,,	12·6 ,,	29368	20/11/30	—	Goat No. 29205 was found dead on 11/12/30. Cause of death unknown. Kid developed no symptoms of Alopecia.

Discussion.

Four pregnant goats were employed in this experiment (Table II), with the result that two typical cases of Alopecia in kids were produced. One animal aborted on the twelfth day of the experiment and one died from an unknown cause three weeks after it had given birth to a normal kid. It will be noticed from Table II that 12.4 Kg. and 23.2 Kg. of the dry plant sufficed to produce the disease, whereas 12.6 Kg. is apparently below the minimum toxic dose necessary for the production of the disease.

EXPERIMENT II.

To determine whether the Colesberg ,, bitterkarroo " (*Chrysocoma tenuifolia* Thunb.) will produce Alopecia in kids.

The plant was collected in the flowering stage during October on the farm Springfontein, Colesberg district. Another attempt was made to persuade the experimental animals to take the plant voluntarily, but this had to be abandoned, as after three days starvation they still refused to take the fresh plant.

Goat 29208.—On each of two consecutive days this animal received 400 grams of the dry plant.

Result.—Within six hours after the second dose the animal exhibited a profuse diarrhoea associated with pronounced straining, complete inappetence, and drowsiness. An almost hairless kid was aborted with the result that the animal had to be discharged from this experiment. Respiration and pulse accelerated. On each of three consecutive days she received a mixture of 200 c.c. of limewater and 100 c.c. of raw linseed oil, and had completely recovered within a week. The total quantity of dry plant received was 800 grams.

Goat 29210.—Received 400 grams of the dry plant on each of two consecutive days.

Result.—Within six hours after the second dose a profuse diarrhoea set in. Tympanites, apathy, salivation, straining, and an accelerated pulse and respiration were the most outstanding symptoms. A mixture of 200 c.c. of limewater and 100 c.c. raw linseed oil was of no avail, death occurring within sixty hours afetr the commencement of the experiment. The total quantity of dry plant received was 800 grams.

Post-mortem Appearances.—General cyanosis; numerous subepicardial haemorrhages; heart in diastole and the ventricles and auricles distended with coagulated blood; hyperaemia and oedema of the lungs; hyperaemia of the liver; atrophy of the spleen; an acute catarrhal gastro-enteritis. The uterus contained an almost mature foetus.

Goat 29219.—This animal, after having received 400 grams of the dry plant on each of two consecutive days, developed the above described symptoms and died within 40 hours after the commencement of the experiment. The total quantity of dry plant received was 800 grams.

Post-mortem Appearances.—General cyanosis; heart in diastole and both ventricles distended with coagulated blood; numerous sub-endocardial haemorrhages in left ventricle; hyperaemia and slight oedema of the lungs; hyperaemia of the liver; haemorrhages in the abomasum; an acute catarrhal enteritis.

The uterus contained an almost mature foetus.

As the Colesberg " bitterkarroo " proved to be more toxic than the Willowmore specimen, the following pregnant goats were drenched with smaller quantities of the plant.

Goat 29206—

6/11/30—200 grams of the dry plant.

7/11/30—200 grams of the dry plant.

8/11/30—Pronounced diarrhoea and other symptoms as previously described. Treated with raw linseed oil and limewater.

9/11/30—Marked improvement. Again treated.

10/11/30—Completely recovered.

11/11/30–16/11/30—50 grams of dry plant daily.

16/11/30—Twin kids born (Nos. 29357 and 29359).

17/11/30—100 grams of the dry plant.

18/11/30—100 grams of the dry plant.

19/11/30—Slight diarrhoea; inappetence and apathy; 100 grams of the dry plant.

20/11/30—Profuse diarrhoea; treated with raw linseed oil and limewater as previously described.

21/11/30—Recovering.

22/11/30—Completely recovered; 100 grams of the dry plant.

23/11/30–25/11/30—100 grams of the dry plant daily.

The animal was in a poor condition with a consequent low milk-yield with the result that she had to be discharged from the experiment.

The total quantity of dry plant received was 1,400 grams in the course of nineteen days.

Kid 29357.—This animal, together with kid 29358, had to be partly hand-reared as their mother (goat 29206) had very little milk. As was expected on account of the little mother's milk obtained, this kid developed no symptoms of Alopecia.

Kid 29358.—It died within twenty-four hours after birth, the post-mortem revealing an acute catarrhal duodenitis and jejunitis.

Goat 29211—

6/11/30—200 grams of the dry plant.

7/11/30—200 grams of the dry plant.

8/11/30—Diarrhoea with the accompanying symptoms.

9/11/30} Treated with raw linseed oil and limewater as pre-
10/11/30} viously described.

11/11/30—Completely recovered.

11/11/30–5/12/30—50 grams of the dry plant daily.

6/12/30—Gave birth to a normal kid (No. 29374); 300 grams of the dry plant.

8/11/30—300 grams of the dry plant.

9/11/30—400 grams of the dry plant.

10/12/30—400 grams of the dry plant.

11/12/30—Pronounced diarrhoea with the accompanying symptoms; received a mixture consisting of 40 c.c. raw linseed oil, 100 c.c. limewater and 1 gram tannic acid.

12/12/30—Marvellous improvement.

13/12/30—Marvellous improvement.

14/12/30—Completely recovered.

15/12/30—300 grams of the dry plant.

17/12/30—Slight diarrhoea.

18/12/30—Slight diarrhoea; 200 grams of the dry plant.

19/12/30—Slight diarrhoea.

20/12/30—300 grams of the dry plant.

22/12/30—300 grams of the dry plant.

23/12/30—300 grams of the dry plant.

24/12/30—300 grams of the dry plant.

27/12/30—300 grams of the dry plant.

29/12/30–5/1/30—Daily 300 grams of the dry plant.

The animal received a total of 6.7 kilograms of the dry plant in the course of two months.

Kid 29374.—It developed no symptoms of Alopecia.

Goat 29212—

6/11/30—200 grams of the dry plant.

7/11/30—200 grams of the dry plant.

8/11/30—Diarrhoea with its accompanying symptoms; treated with a mixture of raw linseed oil, limewater and tannic acid as described before.

9/11/30—Improved: again treated.

10/11/30—Improved; again treated.

11/11/30—Complete recovery.

11/11/30–30/11/30—50 grams of the dry plant daily.

30/11/30—Gave birth to a normal kid (No. 29370).

1/12/30—150 grams of the dry plant.

2/12/30—150 grams of the dry plant.

3/12/30–10/12/30—300 grams of the dry plant daily.

11/12/30—Pronounced diarrhoea with its accompanying symptoms; treated with a mixture of raw linseed oil, limewater and tannic acid.

12/12/30—Marked improvement.

13/12/30—Improving.

14/12/30—Complete recovery, but poor in condition with a consequent low milk yield.

As this animal was in such a poor condition it was decided to discontinue the drenching. It received 3.95 kilograms of the dry plant in the course of thirty-five days.

Kid 29370 (born on 31/11/30).—On the 13/12/30 the kid developed a pronounced diarrhoea and showed apathy and inappetence. From 15/12/30 rapid improvement set in until complete recovery on 17/12/30.

No symptom of Alopecia appeared.

Goat 29218—

6/11/30—200 grams of the dry plant.

7/11/30—200 grams of the dry plant.

8/11/30—Pronounced diarrhoea and listlessness, pulse extremely accelerated and weak; respiration hurried.

9/11/30—Died previous night.

Post Mortem Appearances.—Advanced decomposition; an acute catarrhal gastro-enteritis; an almost full-grown foetus in uterus.

Result.

Seven pregnant goats were engaged in this experiment (Table III). The Colesberg "bitterkarroo" proved to be much more toxic than the Willowmore variety, with the result that one animal aborted and three died. The milk-yield of two of the goats was very low. Unfortunately the conclusions concerning Alopecia in this experiment have to be drawn from one case (No. 29211) only and this was negative.

In the case of Kid 29370 (mother goat 29212) a pronounced diarrhoea developed at the age of twelve days, and had the drenching of its mother not been discontinued from the fourth day after birth, the possibility exists that it would have developed Alopecia, as a diarrhoea precedes the shedding of the hair.

TABLE III (Experiment II).

Dried Colesberg Bitterkarroo.

Goat No.	Date on which Dosage Commenced.	Period of Dosage.	Quantity of Dry Plant Received.	Kid No.	Date of Birth.	Date of Appearance of Alopecia Symptoms.	Remarks.
29208	3/11/30	2 days	800 grams	—	—	—	Developed a profuse diarrhoea on 4/11/30, and aborted on 10/11/30. Recovered after treatment.
29210	3/11/30	2 days	800 grams	—	—	—	Profuse diarrhoea on 4/11/30, and died during the night of 5/11/30.
29219	3/11/30	2 days	800 grams	—	—	—	Profuse diarrhoea on 4/11/30, and died during the night of 4/11/30.
29206	6/11/30	19 days	1·4 Kg.	29357 29358	16/11/30	—	A profuse diarrhoea developed after the animal had received 200 grams of the plant on two consecutive days. After treatment with limewater and raw linseed oil recovery took place. Subsequently the animal received 1,000 grams as described in the experiment. One of the kids died on 17/11/30, and the other one had to be discharged as the mother had no milk.
29211	6/11/30	60 days	6·7 Kg.	29374	6/12/30	—	The mother developed a diarrhoea which disappeared after treatment. The kid developed no symptoms of Alopecia up to 5/1/31.
29212	6/11/30	35 days	3·95 Kg.	29370	30/11/30	—	Both mother and kid developed a diarrhoea. No symptoms of Alopecia appeared.
29218	6/11/30	2 days	400 grams	—	—	—	On 8/11/30 the animal developed a pronounced diarrhoea and died during the night of 9/11/30.

EXPERIMENT III.

To ascertain whether it is possible to produce Alopecia in kids by drenching their mothers from the day of parturition.

Goat 29215—Kidded in 13/11/30 (Kid No. 29355). From 13/11/30 to 8/12/30 this animal received 11.6 kilograms of the dry Willowmore bitterkarroo.

Kid 29355.—It developed no symptoms of Alopecia.

Goat 29209.—Kidded on 15/11/30 (Kid No. 29356). From 15/11/30 to 9/12/30 this goat received 11.2 kilograms of the dry Willowmore bitterkarroo.

Kid 29356.—It developed no symptoms of Alopecia.

Result.

From the above it would appear that for the production of Alopecia in kids it is necessary that they be exposed to the effects of the toxin during a part of their intrauterine life. This point will be discussed at the conclusion of this article.

EXPERIMENT IV.

To determine whether Alopecia can be produced in kids by drenching them with the Willowmore bitterkarroo.

Kid 29246 (*Mother* 29216).—This animal, which was born on 1/11/30, received the following amounts of the dry plant.

5/11/30–8/11/30—10 grams daily.
10/11/30—15 grams daily.
14/11/30—20 grams.
15/11/30—20 grams.
17/11/30—25 grams.
18/11/30—25 grams.
19/11/30—30 grams.
20/11/30—30 grams.
21/11/30—35 grams.
22/11/30—40 grams.
24/11/30—45 grams.
25/11/30—50 grams.
26/11/30—50 grams.
27/11/30—60 grams.
28/11/30—80 grams.
29/11/30—90 grams.
1/12/30—100 grams.
2/12/30—100 grams.
3/12/30–8/12/30—120 grams daily.

It, therefore, received 1.62 kilograms of the dry plant in the course of thirty-four days.

Result.

Complete negative.

Kid 29361 (*Mother* 29217). It was born on 15/11/30 and was drenched with the following amounts of the dry Willowmore bitter-karroo.

19/11/30—10 grams.
20/11/30—10 grams.
21/11/30—15 grams.
22/11/30—20 grams.
24/11/30—25 grams.
25/11/30—30 grams.
26/11/30—30 grams. Slight diarrhoea.
27/11/30—30 grams. Slight diarrhoea.
28/11/30—40 grams. Diarrhoea improving.
29/11/30—50 grams. Diarrhoea worse.
1/12/30—60 grams. Diarrhoea improving.
2/12/30—60 grams. Diarrhoea improving.
3/12/30—80 grams. Complete recovery.
4/12/30—80 grams, Vomited after drenching.
5/12/30—100 grams. Vomited after drenching.
6/12/30—80 grams. Vomited after drenching.
8/12/30—80 grams. Vomited after drenching.
9/12/30—80 grams. Vomited after drenching.
10/12/30—80 grams. Vomited after drenching.
11/12/30—100 grams. Vomited after drenching.
12/12/30—100 grams. Vomited after drenching.
13/12/30—100 grams. Vomited after drenching.
15/12/30—100 grams. Vomited after drenching.

This kid received 1.36 kilograms of the dry plant during a period of twenty-six days without developing any symptoms of Alopecia, the only noticeable ill-effect being a transitory diarrhoea. Emesis was due to the large amount of fluid given.

Result.

None of the kids developed any symptoms of Alopecia, in spite of the fact that they received maximum amounts of the dry plant over a long period.

EXPERIMENT V.

To ascertain whether Merino lambs are susceptible to Alopecia.

For this purpose four pregnant Merino ewes were drenched with the dry Willowmore "bitterkarroo." The dry plant as such was offered to the sheep, but was refusd. Small quantities were then mixed with dry lucerne hay, but still the animals bluntly refused to take any of the mixture. Hence is was resorted to drenching.

As nothing was known about the effects of the plant on Merino sheep, is was decided first to determine these before commencing the actual experiment. To this end sheep 38412 was drenched with the dry plant.

Sheep 38412—

13/10/30—400 grams.

14/10/30—400 grams.

15/10/30—400 grams.

16/10/30—400 grams in the morning and 400 grams in the afternoon.

17/10/30—400 grams in the morning and 400 grams in the afternoon.

18/10/30—Slight diarrhoea. 400 grams.

20/10/30—400 grams in the morning and 400 grams in the afternoon. Slight diarrhoea.

21/10/30—400 grams; diarrhoea; listlessness; accelerated pulse and respiration; thirst.

22/10/30—400 grams in the morning and 400 grams in the afternoon; diarrhoea pronounced.

23/10/30—Pronounced diarrhoea and straining; general weakness; pulse extremely accelerated; respiration hurried; losing condition; drenching discontinued.

These symptoms culminated in death during the night of 25/10/30.

Post-mortem Appearances.—Decomposition too advanced to discern any definite lesions.

Result.

The animal developed a slight diarrhoea after having received 2.8 kilograms of the dry plant in the course of five days. The doses for the pregnant animals were based on the effects of the above quantities of the plant on this sheep. In order to be able to draw definite conclusions from the results of this exeriment the ewes will have to receive maximum quantities of the plant. As the above sheep apparently tolerated 2.8 kilograms of the dry plant given in five days with very slight ill-effects, it was decided to give the pregnant ewes 400 grams daily.

Ewe 29199—

27/10/30—400 grams.

28/10/30—400 grams.

29/10/30—400 grams. Gave birth to a normal lamb (No. 29243)

30/10/30—400 grams.

31/10/30—400 grams in the morning and 400 grams in the afternoon.

1/11/30—400 grams; slight diarrhoea in the afternoon.

2/11/30—Diarrhoea; salivation; pronounced thirst; straining; accelerated pulse and respiration.

3/11/30—Died.

Post-mortem Appearances.—General cyanosis; numerous subepicardial haemorrhages; degeneration of myocard; hyperaemia and slight oedema of the lungs; pronounced degeneration of the liver and kidneys; atrohy of the spleen; a pronounced acute catarrhal enteritis affecting the whole of the small and big intestine.

Lamb 29243 (*born on* 29/10/30.—It was suckled by its mother for five days only and as a result of her death it had to be fed on cow's milk.

Result.

No symptoms of Alopecia developed.

Ewe 29196—

29/10/30–4/11/30—400 grams daily.

3/11/30—Gave birth to a normal lamb (No. 29242).

5/11/30—Slight diarrhoea; treated with a mixture of limewater and raw linseed oil.

6/11/30—Diarrhoea; treated as above.

7/11/30—Recovered.

8/11/30—200 grams.

10/11/30—Diarrhoea; limewater and raw linseed oil.

11/11/30—Diarrhoea improving, but condition poor.

12/11/30—Milk-yield very low; condition poor.

13/11/30 }
14/11/30 } Milk-yield very low; condition poor.

This animal, with its lamb, No. 29242, were discharged on 17/11/30, as it had very little milk. On the morning of the 19/11/30 it was found dead in the stable.

Post-mortem Appearances.—Anaemia; cachexia; hyperaemia; oedema and emphysema of the lungs, with a large amount of coagulated blood in the bronchi and trachea; heart in systole.

Lamb 29241 (*born on* 3/11/30).—As its mother (Ewe 29196) had very little milk, it had to be partly hand-reared.

Result.

This animal developed no symptoms of Alopecia.

Ewe 29197—

29/10/30–4/11/30—400 grams daily.

3/11/30—Gave birth to a normal lamb (No. 29241).

5/11/30—Slight diarrhoea; treated with a mixture of limewater and raw linseed oil.

6/11/30—Diarrhoea; treated as above.

7/11/30—Diarrhoea; treated as above.

8/11/30—Pronounced diarrhoea; vomiting; apathetic; accelerated and weak pulse; hurried respiration.

Died at 9 a.m.

Post-mortem Appearances.—General cyanosis; marked hydrothorax; both ventricles of the heart distended with coagulated blood; hyperaemia and oedema of the lungs with ruminal contents in the trachea and bronchi; slight haemonchosis; an acute catarrhal enteritis affecting both the small and big intestine.

Histology.—Heart, liver, spleen and kidneys: Negative.

Lamb 29241.—Died from a bilateral acute lobar pneumonia during the night of 13/11/30 without having developed any symptoms of Alopecia.

Ewe 29195—

29/10/30–4/11/30—400 grams daily.

4/11/30—Gave birth to a normal lamb. Animal was discharged from the experiment as it had no milk.

On the 6/11/30 it developed a diarrhoea and in spite of treatment with limewater and raw linseed oil it died during the night of 7/11/30.

Post-mortem Appearances.—General syanosis; hyperaemia and oedema of the lungs; slight hydroperitoneum and hydrothorax; both auricles and ventricles of the heart distended with coagulated blood; atrophy of the spleen; acute catarrhal abomasitis duodenitis, typhlitis and colitis; a croupous jejunitis.

Histology.—Liver, heart, spleen and kidney: Negative.

Lamb (died before it was numbered).—It was found dead on the morning of 6/11/30 and the post-mortem revealed an acute catarrhal gastritis.

Result.

The results of this experiment as far as Alopecia is concerned are inconclusive, owing to the fact that the quantities of the plant given had pronounced toxic effects on the sheep, with the result that they either had very little or no milk for the lambs or died. The dry plant proved to more toxic to sheep than to goats.

TABLE IV (Experiment V).

The Effect of Willowmore "Bitterkarroo" on Merino Ewes and their Lambs.

Ewe No.	Date on which Dosage Commenced.	Period of Dosage.	Quantity of Dry Plant Received.	Lamb No.	Date of Birth.	Date of Appearance of Alopecia Symptoms.	Remarks.
29199	27/10/30	6 days	2·8 Kg.	29243	29/10/30	—	On 1/11/30 the ewe developed a diarrhoea and died on 3/11/30. Lamb developed no symptoms of Alopecia.
29196	29/10/30	7 days	2·8 Kg.	29242	3/11/30	—	On 5/11/30 a slight diarrhoea set in, which disappeared after treatment. On 8/11/30 another 200 grams of the plant were given with the result that diarrhoea again developed. On account of the lamb had to be discharged from the experiment on 17/11/30. On the morning of 19/11/30, the ewe was found dead.
29197	29/10/30	7 days	2·8 Kg.	29241	3/11/30	—	On 5/11/30 diarrhoea developed and, in spite of treatment, death occurred on 8/11/30. The lamb died from a bilateral acute lobar pneumonia during the night of 13/11/30, without having developed any symptoms of Alopecia.
29195	29/10/30	7 days	2·8 Kg.	Not Numbered	4/11/30	—	Ewe had no milk and was discharged from the experiment. It developed an acute diarrhoea on 6/11/30, and died during the night of 7/11/30. The lamb was found dead on the morning of 6/11/30.

THE EFFECTS OF POTASSIUM IODIDE ON PREGNANT ANGORA GOATS.

It is of interest to submit on next page a table of the abortions which occurred in the experimental groups of Angora goats receiving potassium iodide. The calculation of the daily doses of potassium iodide for the pregnant goats was based upon the work of Kelly* (1925).

The facts contained in the above table must be viewed in the light of the following information. The total number of pregnant goats employed in Experiment I were twenty-four, and of these eight received potassium iodide. Of these twenty-four animals six aborted, five of which were engaged in the potassium iodide group and one in the salt-bonemeal group. Hence the abortions in the potassium iodide group amounted to 62.5 per cent., whereas in the rest of the animals employed in this experiment these amounted to 6.2 er cent. (that is one in sixteen).

In Experiment II nineteen pregnant goats were engaged and of these seven belonged to the potassium iodide group. Of the seven potassium iodide animals three aborted, whereas in the rest of the animals one abortion occurred in the salt-bonemeal group and one in the Bothablombos group. The abortions in the potassium iodide group therefore amounted to 43 per cent., whereas in the rest of the animals in Experiment II they amounted to 10.5 per cent.

In Experiment III twenty pregnant goats, which received potassium iodide as described before, were allowed to run with the flock of pregnant goats. Of these animals five aborted, the percentage of abortions being 25 per cent.

The twenty pregnant goats receiving 5 grams of sulphur daily, and running with the twenty potassium iodide goats in the same flock, as well as the flock of three hundred pregnant goats, served as controls to the animals receiving potassium iodide. No abortions occurred in the sulphur group, whereas only six occurred in the flock of three hundred pregnant goats.

From the above it is evident that the percentage abortions in the potassium iodide–bitterkarroo group in comparison with the other potassium iodide groups is abnormally high. As the bitterkarroo itself is inclined to produce abortions this high percentage is quite conceivable.

In regard to the abortions in the flock cotyledonosis must be taken into consideration.

* Kelly, F. C. (1925): The Influence of Small Quantities of KI on the Assimiliation of N,P, and Ca in the Growing Pig. *B. Jnl.*, 19, 559-568.

TABLE V.

Effects of Potassium Iodide on Pregnant Angora Goats.

Goat No.	Amount of Potassium Iodide per Day.	Period of Dosage.	Total Amount of Potassium Iodide Received.	Additional Treatment.	Date Dosage with Potassium Iodide was Commenced.	Date of Abortion.
	Grams.	Days.	Grams.			
1	0·1	8	0·8	Ingested daily approximately 4 lb. of bitterkarroo per head (figures from the Willowmore Experiment I).	12/8/30	19/8/30
2	0·1	9	0·9		12/8/30	20/8/30
3	0·1	12	1·2		12/8/30	23/8/30
4	0·05	16	0·8		1/9/30	16/9/30
5	0·05	16	0·8		1/9/30	16/9/30
6	0·1	10	1·0	Ingested daily approximately 2 lb. of flowers and leaves of Bothablombos (figures from the Willowmore Experiment II).	12/8/30	21/8/30
7	0·1	12	1·2		12/8/30	23/8/30
8	0·05	16	0·8		1/9/30	16/9/30
9	0·1	17	1·7	These animals belong to the group of 20 pregnant goats which were running with the remainder of the flock (figures from the Willowmore Experiment III).	12/8/30	28/8/30
10	0·1	20	2·0		12/8/30	1/9/30
11	0·1 gm. from 12/8/31–31/8/30 0·05 gm. from 1/9/30–11/9/30	31	2·55		12/8/30	11/9/30
12	0·1 gm. from 12/8/30–31/8/30 0·05 gm. from 1/9/30–14/9/30	34	2·7		12/8/30	14/9/30
13	0·1 gm. from 12/8/30–31/8/30 0·05 gm. from 1/9/30–14/9/30	34	2·7		12/8/30	14/9/30

DISCUSSION.

Cause of Alopecia.

It has been definitely proved both during the course of field experiments in the Willowmore district and at Onderstepoort that *Chrysocoma tenuifolia* is the cause of Alopecia in kids and the lambs of cross-breeds of sheep.* Furthermore, it has been shown that the sun-dried plant is also capable of causing the disease. In the experimental animals the earliest case of Alopecia occurred in a three-day-old kid and the latest case in an eleven-day-old kid.

Prevention.

In order to prevent the disease it is obvious that the pregnant and suckling animals should be kept away from veld where there is luxurious growth of the "bitterkarroo." On farms where the mountain grazing is of a good quality, and where there is a sufficient water supply for the growing of green foodstuffs, this method of preventing the disease can be applied with great success. The position naturally is different on farms where the above conditions do not exist. It is on the latter farms that the owners have completely stopped kidding and lambing, as they suffered losses up to 100 per cent.

It has been previously mentioned that many farmers combat the disease with great success by removing the pregnant animals from "bitterkarroo-veld" fourteen days before kidding and lambing and not returning them to such veld before the kids and lambs are fourteen days old There is no experimental evidence to bear this out, but both from field observations and experimental results it appears that after the age of fourteen days kids and lambs will not, or very rarely, develop the disease. Very few cases are known where three and four weeks' old kids and lambs suffered from Alopecia.

Another method practised by farmers with a fair degree if success in preventing Alopecia is the partly milking of the suckling goats and ewes. This milk is taken by the natives without any deleterious effects.

The Susceptibility of the Young of Different Species of Stock to Alopecia.

Up to the present Alopecia has been known to occur only in kids, and lambs of Blackhead Persians and all cross-breeds if sheep. No cases have been reported in Merino lambs or any other secies of domestic animals, except those mentioned by Van Rensburg (1925). Nothing definite can be said concerning the susceptibility of Merino lambs to Alopecia, as no Merino sheep are kept in the Alopecia areas. The results of the experiment conducted at Onderstepoort to determine the susceptibility of Merino lambs to Alopecia are inconclusive.

* This plant is ingested by the pregnant and lactating animals and the "toxin" producing Alopecia eliminated in the milk, which in turn is ingested by the kids and lambs. It is in this way that the latter animals contract Alopecia.

The Susceptibility of Full-grown Sheep and Goats to the Effects of the Bitterkarroo.

Merino ewes proved to be more susceptible than Angora goats. A noteworthy fact is that in the course of the Willowmore experiments it was found that when the fresh flowering " bitterkarroo " alone was fed to the Angora goats at the rate of four pounds per head per day the animals developed a fatal diarrhoea, whereas the same amount of plant, when supplemented by lucerne hay and green barley, had no ill-effects on the animals with the exception of loss in condition. It might be stated here that at Onderstepoort excellent results were obtained by treating the diarrhoea with a mixture of 100 c.c. raw linseed oil, 100 c.c. limewater and 1.0 gram tannic acid. Both at Willowmore and at Onderstepoort a large number of animals, which suffered from diarrhoea caused by the bitterkarroo, were treated with limewater and raw linseed oil and with a mixture of limewater, raw linsed oil and tannic acid and the latter mixture was found to be far superiod to the former.

The Direct Effects of the " Bitterkarroo " on Kids.

Two four-day-old kids were drenched daily with comparatively large amounts of the dry plant without any apparent ill-effects, one of the kids showing a slight transient diarrhoea. No symptoms of Alopecia developed. From these results it appears that either (*a*) the " Alopecia-toxin " is not present as such in the plant,but has to be formed or modified by the mother goat or ewe, or (*b*) for the appearance if Alopecia it is essential that the " Alopecia-toxin " must act on the foetus for a certain period, or (*c*) the amount of plant given, although maximum quantities were administered, was too small. In the last case it must be remembered that the mother-goats consumed approximately four pounds of the fresh plant per head per day and the possibility exists that the " Alopecia-toxin " might be excreted in the milk in a concentrated solution. It is obvious that such enormous amounts of plant cannot be administered to kids and lambs.

In this connection it might be stated that two goats were drenched for twenty-five days with the dry plant from the day they kidded with completely negative results.

The Relative Toxicity of the Colesberg and Willowmore " Bitterkarroo " to Full-grown Angora Goats.

Both varieties of the plant were utilised in the dry state and in the flowering stage. The Colesberg variety proved to be much more toxic than that obtained from Willowmore. The only noticeable difference in the two varieties was that the Colesberg plant showed a much more luxuriant growth than the Willowmore variety. The rainfall three months prior to the collection of the plant was much higher in the Willowmore than in the Colesberg district. The soil on which the " bitterkarroo " was collected in the Colesberg district is of a much superior quality than that of the farm on which the Willowmore plant was collected.

The Relation between Alopecia and the Diarrhoea.

In a number of cases of Alopecia in kids and lambs the Alopecia was preceded by a diarrhoea, but in the great majority of cases, Alopecia was the first noticeable symptom. Apparently the cause of the diarrhoea in the kids and lambs is a two-fold one, namely, a diarrhoea caused by a toxin contained in the " bitterkarroo " plant and excreted in the milk and one caused by the mechanical irritation of the ingested hair. After the appearance of Alopecia these two factors will act as synergists. Most probably the above toxin is also responsible for the diarrhoea in the full-grown goats and sheep. Whether this " diarrhoea-producing toxin " is identical with the " Alopecia-producing toxin " is an open question. The " diarrhoea-producing toxin " complicates the Alopecia experiments, as large amounts of the plant have to be given in order to produce the disease. and on the other hand a sub-toxic dose must be given, as it is essential for the suckling sheep and goats to have a high milk-yield.

POTASSIUM IODIDE AND ITS RELATION TO ABORTION.

A summary of the abortions in all the different experiments at Willowmore and in the controls is given in Table VI.

CONCLUSIONS.

(1) *Chrysocoma tenuifolia* Berg. (bitterkarroo "), has been established as the cause of Alopecia in kids and lambs, and that the " toxin " is eliminated through the milk.

(2) The disease can be successfully prevented by avoiding *Chrysocoma tenuifolia* veld for a period of fourteen days prior to and after kidding and lambing.

(3) Up to the present Alopecia has been known to occur in the kids of all breeds of goats and all cross-breeds of sheep. Cases in Merino sheep have been reported.

(4) *Chrysocoma tenuifolia* when ingested in large quantities produces abortions in pregnant sheep and goats as well as symptoms of severe gastro-intestinal irritation.

(5) Two four-day old Angora kids drenched with large amounts of *Chrysocoma tenuifolia* for a period of twenty days developed no symptoms of Alopecia.

(6) The Colesberg *Chrysocoma tenuifolia* has been found much more toxic to sheep and goats than the Willowmore variety.

(7) A point to be investigated is the relation between the " diarrhoea-toxin " and " Alopecia-toxin " contained in *Chrysocoma tenuifolia*.

(8) The abortions in the goats receiving potassium iodide amounted to 37 per cent., whereas in the rest of the experimental animals and the controls they amounted to 2.3 per cent. only.

TABLE VI.

The Effect of Potassium Iodide on Pregnant Angora Goats.

Expt. No.	Number of Goats in Experiment.	Number of Goats which Received KI.	Total Number of Goats which Received KI.	Number of KI. Goats which Aborted.	Total Number of KI. Goats which Aborted.	Total Number of Goats in the Experiment + Controls.	Percentage Abortion in KI. Groups.	Percentage Abortions in Rest of Experimental Goats and Controls.
I	24	8	35	5	13	383	37	2·3
II	19	7		3				
III	20	20		5				
IV	20	None (all received sulphur)		None.				

From the above table it is evident that the abortions in the potassium iodide groups amounted to 37 per cent., whereas in the rest of the experimental animals and controls these amounted to 2.3 per cent. only.

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In conclusion I would like to thank Dr. du Toit, Director of Veterinary Services, and Dr. de Kock, Deputy Director of Veterinary Services, for reading through my article and making valuable suggestions; also Mr. Meyer, photographer at Onderstepoort, for preparing the attached photographs.

LITERATURE.

Reko (1928) described a disease in horses, mules, cattle, sheep and goats occurring in the southern parts of the United States of America, in the course of which the animals shed their coat. In advanced cases of this disease there is pronounced inflammation of the joints, which ultimately causes death.

Stock-owners incriminate the " loco-weed " (*Astragalus lambertii, Aragalus molissimus* and *Cystium diphysium*). Reko has not seen the disease where *Astragalus* sp. do not occur.

A similar disease occurs in Mexico in animals which ingest *Tamarindus indica* over long periods. As soon as the animals are prevented from feeding on the plant the hair commences growing. The aborigines of South Mexico daily partake of the ground Tamarindus seeds to depilate themselves.

These seeds are used to breed the small hairless Chihuahua dogs. Pneumonia and dropsy frequently are the cause of death in these hairless animals.

Furthermore, thallium salts are active poisons and may lead to Alopecia (Ward, 1930). Since 1930 thallium has been used in America as a proprietary rat-poison and thallium poisoned grain for the control of prairie dogs, with the result that many losses due to the ingestation of this poisoned grain have occurred in sheep. It was found that sub-lethal doses of thallium down to 9 milligrams per Kg. body weight cause Alocepia in sheep.

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Fig. I.—Natural case of Alopecia.

Fig. II.—Natural case of Alopecia, showing almost complete hairlessness. The skin appears quite normal.

Fig. III.—Animal in Fig. II subsequently developed an acute crustaceous dermatitis.

Fig. IV.—A kid recovering from Alopecia. A new coat is appearing.

Fig. V.—Kid 29371. A case of Alopecia produced at Onderstepoort.

Fig. VI.—Kid 29371. Alopecia. Condition more advanced. Diarrhoea and acute purulent conjunctivitis and rhinitis present.

Fig. VII.—Kid 29371. Alopecia. Dermatitis setting in. Note the bad condition of the animal.

Fig. VIII.—Kid 29371. Showing pronounced diarrhoea and hairless patches on hind legs.

Fig. IX.—(*a*) Ruminal and (*b*) abomasal hairballs. collected from Kid 29371. Natural size.

Section IX.

Animal Industry.

J. E. Duerden and V. Bosman — Fibre Lengths, Thicknesses and Qualities in a Single Wool Staple.

J. E. Duerden and V. Bosman — Staple Length and Crimped and Straight Length of Merino Wool Fibres.

J. E. Duerden and D. G. Bell — Quality, Variation and Distribution in the Fleece of the Merino.

J. E. Duerden and G. S. Maré — Rates of Growth of Merino Wool, Month by Month.

D. J. Schutte and C. A. Murray — The Effect of Barley in the Ration of the Quality of Bacon.

D. J. Schutte and G. N. Murray — Planes of feeding for fattening steers.

J. W. Groenewald — The Causes of Variation in the Fat content in Successive Portions of Milk.

Fibre Lengths, Thicknesses and Qualities in a Single Wool Staple.

By Dr. J. E. DUERDEN, Director of Wool Research, and V. BOSMAN, M.Sc., Sheep and Wool Research Officer.

The fleece of the Merino is by no means uniform all over the body. The wool is usually longest and finest over the shoulder and shortest and coarsest over the britch, and the separate or individual fibres comprising the staples differ accordingly. The problem of these variations is of the highest importance in wool production, since the manufacturer desires as great a uniformity as possible. Studies are in progress to provide accurate data as regards the relative length and thickness of the fibres of the different regions. An account is here given of the variability in length and thickness of the fibres to be found in a single staple, particular attention being given to the correlation of the one attribute to the other.

A single staple, measuring $3\frac{3}{4}$ of an inch in length, was shorn from the fleece of a ram and the yolk removed by immersion in ether. One thousand complete fibres were then drawn out at random from the cut end of the staple. As removed, the straight length of each was measured on a scale to within an eighth of an inch, and the fibre then placed in the class or group corresponding with the measurement. The results are given in Table I.

From the table it is seen that 21 classes are represented, each differing by an eighth of an inch, the shortest fibres being 4 inches in length and the longest 6.5 inches, that is, more than one and a half times longer. The greatest number of fibres, 103, appears in class length 5.25 inches, which is the middle or mode of all the lengths. From this a less and less number occurs in each of the classes, passing towards the shorter and the longer ends, and, as shown in Fig. I, they give rise to an almost normal distribution curve.

In a staple of Merino wool it is thus manifest that most of the fibres will be of an average length, but a number will be longer and about the same number will be shorter than this, and all the lengths will grade into one another.

TABLE I.

Fibre Lengths, Thicknesses and Qualities in a Single Wool Staple.

Class Lengths.			No. of Fibres, 1,000.	Mean Thickness.	Quality Number.
$\frac{1}{8}$-inch.	Inch.	Cm.			
32	4·00	10·16	6	16·75μ	90's
33	4·12	10·47	10	16·58μ	,,
34	4·25	10·79	24	17·71μ	80's
35	4·37	11·11	31	17·48μ	,,
36	4·50	11·43	43	17·71μ	,,
37	4·62	11·74	48	18·85μ	70's
38	4·74	12·06	67	19·02μ	66's
39	4·87	12·38	88	19·28μ	,,
40	5·00	12·70	95	19·75μ	,,
41	5·12	13·01	98	20·03μ	64's
42	5·25	13·33	103	20·69μ	,,
43	5·37	13·65	99	21·40μ	60's
44	5·50	13·97	90	21·78μ	,,
45	5·62	14·28	74	22·23μ	,,
46	5·75	14·60	44	22·35μ	,,
47	5·87	14·92	39	22·93μ	,,
48	6·00	15·24	20	26·92μ	56's
49	6·12	15·55	6	26·23μ	,,
50	6·25	15·87	7	23·37μ	58's
51	6·37	16·19	6	26·90μ	56's
52	6·50	16·51	2	25·20μ	58's
Mean.....	—	13·08	—	20·51μ	—
E. of M.....	—	± 0·797	—	±0·0411	—
S.D.....	—	± 3·74	—	±1·93μ	—
C. of V.....	—	9·1%	—	9·4%	—
C. of C.....	—	—	+ 0·96	—	—

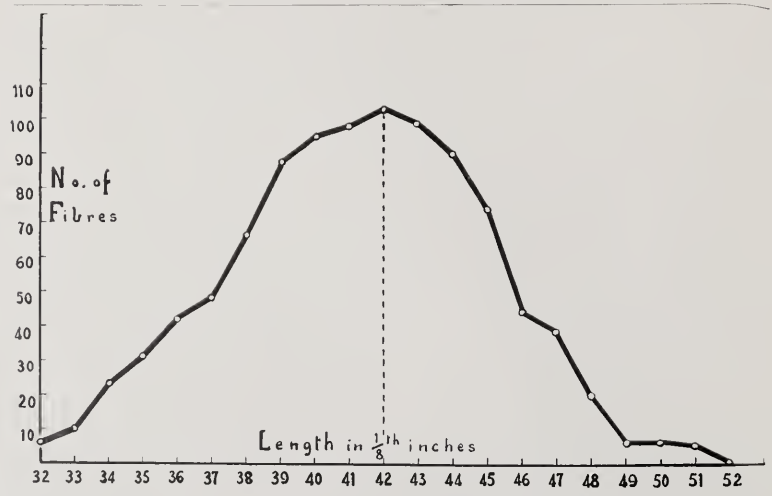

FIG. 1.—Distribution curve of the lengths of the one thousand fibres in Table 1.

The fibres in each class, all of the same length within an eighth of an inch, were next measured as to their thickness. Each group was formed into a strand which was then clipped along its whole length into a large number of small fragments and collected into a tube of ether. Here they were thoroughly intermingled to secure a uniform distribution and afterwards dried and mounted in euparal on a slide, hundreds of fragments being included. Two hundred and fifty thickness measurements of the clippings of each class were then made by means of the Zeiss-Hegener micro-camera and the mean thickness calculated. This is given for each group of fibres in the fifth column.

Apart from a slight irregularity towards each end of the column, due to the small number of fibres within the class, the mean thickness is seen to increase from one end to the other in a regular manner, that is, *the shorter fibres are the finest and the longer the thickest*, the strongest being more than one and a half times thicker than the finest, just as they have been shown to be more than one and a half times longer.

Calculation shows the co-efficient of correlation of length to thickness to be as high as +.96, in other words, *in any staple of wool the fibres increase in thickness according to their length, or, the length varies directly as the thickness.* Were a larger number of fibres taken, a perfect correlation could be obtained, for the methods of measurement adopted obviously provide highly consistent results. Plotting the length and thickness measurements, as in Fig. 2, they are seen to give rise to an almost straight line which would indicate perfect correlation. Norris and Van Rensburg (1930) also state that " Roberts has found that in the lock there is a general relationship between thickness and fibre length ".

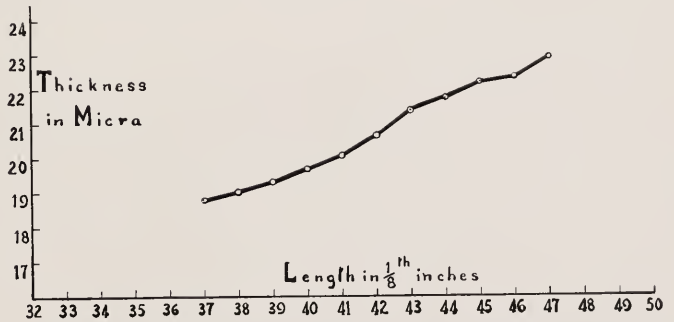

FIG. 2.—Graph showing distribution of the lengths and thicknesses of the fibres in Table 1, the extreme being omitted. An almost straight line is obtained indicating that fibre length and thickness vary directly one with the other.

In a recent paper each of the commercial quality numbers of Merino wool has been assigned its definite thickness range (Duerden, 1929). Applying these to the thickness of the various class lengths, as in the last column, the single staple is seen to contain fibres which as regards thickness represent qualities varying from 90's to 56's,

that is, *a single staple may comprise fibres belonging to practically the whole range of merino wool qualities.* The staple of wool selected would be considered as a 64's quality, yet it contains some fibres as fine as those of a 90's quality wool, and others as strong as a 56's quality, a quality usually considered as below that of merino wools. The greater number of fibres are those of a 64's wool, but along with them are others ranging from 90's to 56's. The shorter fibres being the finer represent the higher qualities, the longer being the stronger are the lower qualities.

For the particular sample studied an almost constant ratio, 1:1.55, exists between the fibre length in centimetres and the thickness in micra for each class, slight departures occurring towards the extremities. This ratio, however, applies only to the particular sample; even on the same sheep, as shown below, staples of similar length may have a different proportional thickness, or when of similar thickness they may have a different proportional length.

Table II gives the fibre lengths and their thickness in four separate staples taken from different regions of the same sheep: shoulder, back, belly and britch. To reduce the labour involved the intervals of the class lengths are much wider than in Table I, namely, one centimetre, hence only four or five classes are represented in place of the twenty-one; also, only one hundred fibres were drawn at random from each staple and distributed according to their class length, while, as before, 250 micro-measurements were taken of the clippings of each class.

The fibres from the shoulder have the greatest mean length (10.4 cm.), but are also the finest (18.43μ); those from the belly are the next in length (10.14 cm.), and are but slightly thicker (19.08μ); from the back they are still shorter (9.6 cm.) and of about the same thickness (18.67μ); while the britch fibres are by far the shortest (6.2 cm.), and are also the thickest (26.56μ).

The co-efficient of correlation between the different lengths and thicknesses of each separate sample is again very high, +.99 in three cases and +.91 in the belly wool, a result partly to be associated with the wide class lengths adopted. The ratio of the mean class length to the mean thickness varies. In the shoulder sample the ratio is 1:1.77, in the back 1:1.94, and in the belly 1:1.88, while in the britch it is 1:4.12. Thus while in each separate sample the high correlation of length and thickness obtained in Table I is maintained, the actual relationship of length and thickness differs in each case. In the shoulder the fibres are the longest and are also the finest; on the britch they are by far the shortest and are also by far the coarsest. The high correlation between the length and thickness of the fibres holds for each particular sample, but the different areas of the sheep have their particular ratio between length and thickness; the thickness is practically the same for the shoulder, belly and back, but the length differs.

In Table III are given the fibre lengths and thicknesses of single staples from the shoulder region of four different fleeces. In the first two the class interval is 1.59 cm. and in the next two 1.27 cm. Here again in each sample a high co-efficient of correlation is characteristic of the length and thickness of the fibres making up the

TABLE II.

Fibre Lengths and Thicknesses in Single Staples from Different Regions of the Same Fleece.

	Shoulder: Staple, 9 cm.			Back: Staple, 8 cm.			Belly: Staple, 8 cm.			Britch: Staple, 5 cm.		
	Class Length, Cm.	No. of Fibres.	Mean Thickness.	Class Length, Cm.	No. of Fibres.	Mean Thickness.	Class Length, Cm.	No. of Fibres.	Mean Thickness.	Class Length, Cm.	No. of Fibres.	Mean Thickness.
	8	8	16·07 μ	8	18	16·50 μ	8	6	17·15 μ	5	26	20·90 μ
	9	13	16·62 μ	9	24	18·02 μ	9	18	17·50 μ	6	36	25·47 μ
	10	24	18·05 μ	10	38	19·05 μ	10	38	18·37 μ	7	30	30·52 μ
	11	37	19·05 μ	11	20	20·27 μ	11	32	20·22 μ	8	8	35·05 μ
	12	18	20·02 μ	—	—	—	12	6	24·30 μ	—	—	—
Mean.....	10·40	—	18·43 μ	9·6	—	18·58 μ	10·14	—	19·08 μ	6·2	—	26·56 μ
E. of M.....	$\pm$0·0783	—	$\pm$0·0823	$\pm$0·0675	—	$\pm$0·0829	$\pm$0·0661	—	$\pm$0·1153	$\pm$0·0613	—	$\pm$0·2955
S.D.....	$\pm$1·16	—	$\pm$1·22	$\pm$1·00	—	$\pm$1·23	$\pm$0·98	—	$\pm$1·71	$\pm$0·91	—	$\pm$4·38
C. of V.....	11·2%	—	6·6%	10·4%	—	6·6%	9·7%	—	8·9%	14·7%	—	16·5%
C. of C.....	+·99$\pm$0·0013			+·99$\pm$0·0013			+·91$\pm$0·0116			+·99$\pm$0·0013		

TABLE III.

Fibre Lengths and Thicknesses in Single Staples of Different Fleeces.

Fleece No.	1.			2.			3.			4.		
	Class Length, Cm.	No. of Fibres.	Mean Thickness.	Class Length, Cm.	No. of Fibres.	Mean Thickness.	Class Length, Cm.	No. of Fibres.	Mean Thickness.	Class Length, Cm.	No. of Fibres.	Mean Thickness.
	6·98	14	22·19 μ	6·98	13	16·15 μ	10·63	26	18·29 μ	9·36	61	27·10 μ
	8·57	51	25·10 μ	8·57	35	17·00 μ	11·90	41	20·77 μ	10·63	26	28·09 μ
	10·16	29	27·67 μ	10·16	40	19·22 μ	13·17	27	24·61 μ	11·90	11	28·00 μ
	11·75	6	28·87 μ	11·75	12	19·42 μ	14·44	6	25·25 μ	13·17	2	28·87 μ
Mean	8·99	—	25·66 μ	9·38	—	18·06 μ	12·06	—	21·42 μ	10·04	—	27·49 μ
E. of M.	$\pm$0·0405	—	$\pm$0·1180 μ	$\pm$0·0587	—	$\pm$0·0857 μ	$\pm$0·0857	—	$\pm$0·1699 μ	$\pm$0·0513	—	$\pm$0·0337 μ
S.D.	$\pm$0·60	—	$\pm$0·75 μ	$\pm$0·87	—	$\pm$1·27 μ	$\pm$0·86	—	$\pm$2·52 μ	$\pm$0·76	—	$\pm$0·50 μ
C. of V.	6·6%	—	6·8%	9·3%	—	7·0%	7·2%	—	11·7%	7·6%	—	1·7%
C. of C.	+·98$\pm$0·0026			+·96$\pm$0·0051			+·99$\pm$0·0013			+·96$\pm$0·0051		

different classes, but the relation of the mean class length to the mean thickness varies. In Fleece 1 the length is 8.99 cm. and the thickness 25.66μ, while in Fleece 2 with the greater length of 9.38 cm. the thickness is only 18.06μ. Also in the other two fleeces the longer fibres in Fleece 3 are finer than the shorter fibres in Fleece 4. Thus from the same region in different sheep the co-efficient of correlation between length and thickness remains practically the same, though the mean length and thickness relationships are not alike.

It is manifest that the wool from different sheep will vary in length according to its period of growth, and the ratio of the mean length to the mean thickness will vary accordingly, but the co-efficient of correlation will remain the same.

On ordinary handling the fleeces of different sheep are found to vary greatly in staple length and thickness, and even on different parts of the same sheep, and the laboratory study shows that the individual fibres composing each staple vary likewise. The expression for this variability, the co-efficient of variability, is in each case dependent upon two factors. Variability in length depends upon the different lengths of the fibres, that is, their range, and also upon their distribution around the mean; similarly, the variability in thickness depends upon the range of thickness and also upon the manner in which the different fibre thicknesses are distributed about the mean.

The co-efficient of variability of length and thickness has an important application in the manufacturing value of a wool; the ideal condition is one in which the fibres are all of the same length and thickness, but this never obtains. As regards any particular area the one has been shown to vary directly as the other; increase in length is accompanied by an increase in thickness. *It follows that a wool very variable in length will also be very variable in thickness, or a wool uniform in length will also be uniform in thickness. In striving for the uniformity of the one attribute the breeder will tend to attain uniformity of the other.*

In Table II, comprising samples from different regions of the same sheep, the percentage of variability is fairly close for both length and thickness, while in Table III, representing samples from different sheep, the difference is more marked.

Direct correlation of length and thickness does not necessarily exist when samples of different types of wool are intermingled and the fibres then distributed into their classes. This is well shown in Table IV representing a mixture of shoulder and britch wool from the same sheep. The means of their class lengths and their thicknesses have a high correlation when taken separately, but when combined the co-efficient of correlation has the value of $-.52$. In the britch the fibres of the class thickness 20.90μ have a class length of 5 centimetres, while in the shoulder those of approximately the same thickness have the class length of 12 centimetres.

In their paper on the inheritance of some wool characters, Davenport and Ritzman (1926) make the following remarks: "When we compare the mean length and the diameters of the wool fibres from the parent breeds, we find the old saying: 'Fine wool tends to run short and coarse wool tends to grow long' is, in general, confirmed.

TABLE IV.

Correlation of Fibre Lengths and Thicknesses in a Mixture of Wool from Shoulder and Briteh.

Shoulder.			Britch.			Shoulder and Britch Combined.		
Class Length, Cm.	No. of Fibres.	Mean Thick-ness.	Class Length, Cm.	No. of Fibres.	Mean Thick-ness.	Class Length, Cm.	No. of Fibres.	Mean Thick-ness.
5	0	—	5	26	20·90 μ	5	26	20·90 μ
6	0	—	6	36	25·47 μ	6	36	25·47 μ
7	0	—	7	30	30·52 μ	7	30	30·52 μ
8	8	16·07 μ	8	8	35·05 μ	8	8	35·05 μ
9	13	16·62 μ	9	0	—	8	8	16·07 μ
10	24	18·05 μ	10	0	—	9	13	16·62 μ
11	37	19·05 μ	11	0	—	10	24	18·05 μ
12	18	20·02 μ	12	0	—	11	37	1·905 μ
—	—	—	—	—	—	12	18	20·02 μ
Mean.....	10·4	18·43 μ	6·2	—	26·56 μ	8·4	—	22·49 μ
E. of M...	±0·0783	±0·0823 μ	±0·0613	—	±0·2295 μ	±0·1125	—	±0·2480 μ
S.D.....	±1·16	±1·22 μ	±0·91	—	±4·38 μ	±2·36	—	±5·20 μ
C. of V....	11·2%	6·6%	14·7%	—	16·5%	28·1%	—	23·1%
C. of C....	+·99±0·0013		+·99±0·0013			−·52±0·0347		

But, if we compare length and diameter in the hybrid wools, the relation is less close. Thus, in the Southdown×Rambouillet hybrids the average diameter decreases in successive generations, but the length increases in F_2 and F_3 above that of F_1. Again in the Hampshire×Rambouillet hybrids the F_3 generation gives the finest wool and also the longest. It appears, then, that there is no necessary physiological dependence of small diameter on shortness. Fineness is, in itself, no necessary barrier to rapid growth or to the production of a reasonable length in staple, within the limits of length represented in our hybrids. Fibre length and fibre thickness are independent variables genetically. The usual association of shortness and fineness probably depends on the fact that fine wool is much more densely placed on the skin than coarse wool, so that an equal rate of growth of the two kinds entails the greater drain on vigour and nourishment of the former. It is a constitutional limitation in capacity for producing the material of wool fibre that causes shorter staple to be associated with denser, finer wool ".

We may thus conclude that for a limited area where the wool is of the same type the individual fibres vary in thickness according to their length, that is, the longer is the fibre the thicker it is. But in different areas and in different sheep the wool is rarely of the same type and, while the high correlation of length and thickness is maintained for each separately, the ratio of the mean length to the mean thickness differs; the thickness and length ratio varies independently in different regions. The fibre within each follicle in

any limited area has its own genetic rate of growth in length and thickness and one varies with the other, but, though still varying one with the other, the genetic rate differs independently over different areas. Only a homogeneous wool, one from a restricted area, will give a high correlation of the two attributes; a low or a negative correlation implies a wool diversified in fibre character, as in a mixture of shoulder and britch wool from the same fleece, or a blend from different fleeces.

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Staple Length and Crimped and Straight Length of Merino Wool Fibres.

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The length of the staple* is an important factor in wool production and concerns the sheep farmer as well as the wool buyer and seller. The farmer regards good length as a desirable attribute in a flock since it gives a greater weight of wool, and the buyer selects largely on length, as for combing or clothing wools. The staple length does not, however, represent the true length of the wool. In the staple the fibres are all in a crimped or wavy condition and necessarily become longer when straightened out. The table below has been prepared to show the difference between the staple length of various wool samples and the average length of the fibres composing them, both in the crimped condition and when straightened.

The measurements have been prepared as follows: Staple length does not admit of accurate estimation since the fibres do not all reach the same level and the outer surface tends to be matted and uneven. Within certain limits, however, a general average can be taken, and the length of the staple then obtained by direct measurement.

The average crimped fibre length has been found by drawing out at random twenty fibres from the lower end of the staple, and then measuring each along the edge of a scale while in the crimped condition, the fibre being held at each end by means of finely pointed, smoothed forceps. Even though stretched on drawing out, a fibre recovers its original crimping when relaxed. The staples selected were all second or later shearings in order not to include any of the new shorter fibres which occur in the lamb's coat.

The straight fibre length is ascertained by pulling upon the crimped fibres held with the forceps until the crimping is just eliminated, but without any stretching of the fibre. With practice it is readily ascertained when this stage is reached, and highly consistent results have been obtained on test measurements being made from the same sample.

* The term staple is sometimes used as referring only to the length of a wool, as in cotton terminology; more usually, however, it applies to the separate, distinct locks, tufts, or bundles of fibres, varying in size and readily isolated on opening-up a fleece.

TABLE 1.

Length Measurements in Centimetres of Fifteen Staples of Merino Wool of Different Qualities, with the average length of the Component Fibres when crimped and when straight; also percentage amount of crimp and ratio of staple length to straight length.

1. Sample Number.	2. Quality Number and Crimps per Inch.	3. Staple Length.	4. Average Fibre Length, Crimped.	5. Average Fibre Length, Straight.	6. Percentage of Crimp.	7. Ratio, Staple Length to Straight Length.
		A.—*Shallow Crimped Wools.*				
3.....	66's; 14–15	10·3	16·46	12·58	20·27	1 : 1·22
4.....	60's; 10–11	9·0	8·85	11·41	28·92	1 : 1·27
7.....	60's; 10–11	5·2	5·51	6·87	24·68	1 : 1·32
10.....	80's; 18–19	6·50	6·87	8·62	25·47	1 : 1·32
2.....	70's; 16–17	6·50	6·84	8·78	28·37	1 : 1·35
	—	7·56	7·70	9·65	25·54	—
		B.—*Half-circle Crimped Wools.*				
13.....	70's; 16–17	5·00	4·86	7·01	44·25	1 : 1·40
14.....	90's; 20–21	5·10	5·51	7·32	32·85	1 : 1·43
5.....	60's; 10–11	5·30	5·30	7·65	44·34	1 : 1·44
15.....	66's; 14–15	7·00	7·76	10·64	37·11	1 : 1·52
9.....	80's; 18–19	6·00	5·99	9·14	52·59	1 : 1·52
6.....	60's; 10–11	5·10	5·07	7·70	51·87	1 : 1·51
1.....	60's; 10–11	6·20	6·51	9·46	45·32	1 : 1·53
8.....	66's; 14–15	7·60	8·16	12·33	51·10	1 : 1·62
	—	5·91	6·14	8·91	44·93	—
		C.—*Deep Crimped Wools.*				
11.....	66's; 14–15	3·00	3·07	5·20	69·38	1 : 1·73
12.....	66's; 14–15	3·00	3·38	6·22	84·02	1 : 2·07
	—	3·00	3·22	5·71	76·70	—

Comparing the measurements in columns three and four it is found that the average length of the separate fibres in the crimped condition does not differ much from the staple length. The average of the fifteen staple lengths is 6.01 cm., while that of the crimped fibres is 6.27 cm. Usually the fibre length is the greater, but in four cases it is less, the outer surface of the staple necessarily comprising only the longest fibres.

As shown in columns three and five the average of the straight length compared with the corresponding staple length, and also with the crimped length of the fibres, is much greater, but varies considerably.

The ratio between the staple and straight length of the fibres is shown in the last column, while in the sixth column is given the percentage of crimp in the fibres. The variations are seen to be largely associated with differences in the type of crimping of the fibres. The increased length in the straight fibres may vary from about one-fifth of the staple length to over four-fifths, that is, in extreme cases the straight length of the fibres may be nearly double that of the staple length.

In a paper, "A Macroscopical Analysis of the Fleeces of Four Romney Rams," Prof. J. F. Wilson (1929) devotes a section to the ratio of staple length to fibre length. As might be expected great differences occur in the wool from different regions of the same sheep, though a closer relationship holds in the samples from the same region of the four rams, the highest ratio being from the thigh and the lowest from the shoulder. Wilson concludes that the data he obtained are in the main inconclusive and inconsistent for comparative purposes.

The present studies show that ratio comparisons between two attributes of a wool, as between length and thickness and length and crimping, are of value where highly similar wools are concerned.

Types of Crimping.

In Merino wools three main types of crimping may be distinguished, though with all intermediate stages (Fig. 1). These may be termed: 1. *Shallow Crimping*; 2. *Half-circle Crimping*; 3. *Deep Crimping*.

1. *Shallow Crimping.*—The crimps are shallow and often irregular and ill-defined, and each half wave or crimp is shorter than would be a half-circle with the fibre axis on which it rests as a diameter.

Shallow crimping is met with in wools lacking in definiteness, the waves being open and poorly defined. Crimping of this type is usually not desired by the breeder nor by the wool buyer. The stapling of such wools is also usually ill-defined and the wool may be mushy.

In their straight condition the fibres of wools of this type are about one and a quarter to one and a third times longer than in their crimped state or than their staple length.

2. *Half-circle Crimping.*—Each half wave or crimp has approximately the form of a half-circle.

This type of crimping is usually well-defined and even, and indicates a wool of the highest Merino type; it is, perhaps, the most frequent type of crimping and the most desirable for the breeder and for the wool buyer.

When straightened out the separate fibres are about one and a half times longer than in their crimped state or than the length of the staple, i.e., they have the same relationship as half the ratio of a circle to its diameter, $\frac{\pi}{2}$.

3. *Deep Crimping.*—The separate half waves or crimps are longer than would be a half-circle with a diameter corresponding with the length of the axis on which it rests, and are well-defined and even.

This type of crimping is met with in belly wool, also in what is known as watery wool, and in wool lacking compact stapling, but is not of frequent occurrence compared with shallow and half-circle crimped wools. It is not desired by the breeder nor by the manufacturer.

When straightened the separate fibres of over-crimped wools are from one and three quarters to double the length of the fibre in the crimped state or in the staple.

In an earlier paper (1929) it has been shown that in the majority of cases a close correlation exists between the number of crimps in a wool and its thickness or quality, and that the trade practice of estimating quality number mainly by the crimps receives general confirmation.

But the form of the crimping has no direct relationship to the number of crimps to the inch nor to the quality number of the wool, for in the table similar qualities, estimated on their number of crimps, appear under each of the three types. All three crimp types may occur in fine, medium or strong wool.

In practice the form and regularity of the crimps are held to be important indications of the merits of a wool, and an even, well-defined type is highly desired, one in which the crimps return to their original form when the fibre is stretched and then relaxed. What are the actual physico-chemical factors associated with the production of the crimp form cannot be stated until more is known of the method of formation of crimps generally (Barker and Norris, 1930).

Crimps and Fibre Lengths.

Miss Mabel H. Norris and Mr. P. J. J. van Rensburg (1930) have made a study of the total number of crimps in groups of about 100 fibres taken from different locks of Merino and Romney wool, and arrive at the conclusion that: "The number of crimps per fibre, in each lock, is found to be entirely independent of fibre length, and remains approximately constant for fibres of all lengths. . . . It is concluded, therefore, that in the case of Merino wool, crimp formation is a periodic function of time and is independent of the rate of growth of the fibre." In an individual sample of wool a finer fibre will be shorter but will have the same number of crimps as a longer, thicker fibre; it will have closer crimps, that is, more per inch than the longer fibre.

The above relationship only holds when restricted homogeneous samples of wool are under consideration, or wools of altogether similar character. Admixtures of dissimilar wools, as from the shoulder and britch, would not conform to the rule. Taken separately the relationship of the average fibre length and average number of crimps will differ in the two areas.

Correlation of Fibre Length, Fibre Thickness and Crimping.

In a uniform staple it has been found that fibre length and thickness are directly correlated; that is, the longer the fibre the

thicker it is. One thousand fibres from a staple have been grouped according to their length in classes, numbering twenty-one, and the thickness of each class measured. In each instance practically the same ratio of length to thickness is obtained, namely 1 : 1.55, the coefficient of correlation of the whole being + .96. Fibre length has, however, been shown above to be correlated with number of crimps; where uniform staples are concerned the longer the fibre the fewer are the number of crimps per inch. Crimping is, therefore, correlated both with fibre length and with fibre thickness, the latter relationship having been also established from direct studies of crimp and thickness (Duerden, 1929). The three attributes—length, thickness, and crimps—are the most important factors in estimating the spinning value of a wool, and are all shown to be connected in so far as the wools are of similar character.

Length, thickness and crimping may, however, vary independently of one another both in the same fleece, as between shoulder and britch, and in different fleeces; ratios established for one sample are not likely to hold for another sample. A fleece as a whole is too independently variable for ratio comparisons between any two attributes to hold beyond very restricted areas, apart from those of thickness and crimping; moreover, it is possible that similar ratios might be obtained from wools of highly diverse character.

Staple and Fibre Crimping.

In wool practice crimping has a significance only in connection with a wool or a staple as a whole, and in a well-defined staple the crimp formation appears uniform for the whole length. But it is manifest from the above that the crimping of the individual component fibres is not the same, for in the staple will be fibres of different lengths, each with a different number of crimps per unit length. How the composite uniform crimping of the staple results from so many different crimp components is not clear. The crimping of the staple must be regarded as a resultant of that of all the fibres of the staple. Two factors are evidently concerned: the one producing the crimping of the individual fibre, which is probably formative and associated with the follicle, and the other producing the uniform composite staple from the varied elements, which is probably mechanical in nature.

The fibres making up a uniformly crimped strand, a small portion of a staple, are seen to be disposed irregularly with regard to one another, with but little parallelism. Drawn out as individual fibres the crimps of one do not necessarily conform with those of another.

It is clear that the same total number of crimps and of the same form can occur in a short fibre as in a long fibre, and would, therefore, give the same ratio between the crimped length and the straight length for both fibres. Where the same ratio does not obtain it manifestly represents a difference in the form of the crimps. As shown in Table 2 various ratios obtain in the individual fibres from a sample, although the same number of crimps occurs in each; hence we may conclude that the crimp form differs, though collectively giving a uniform crimping in the staple.

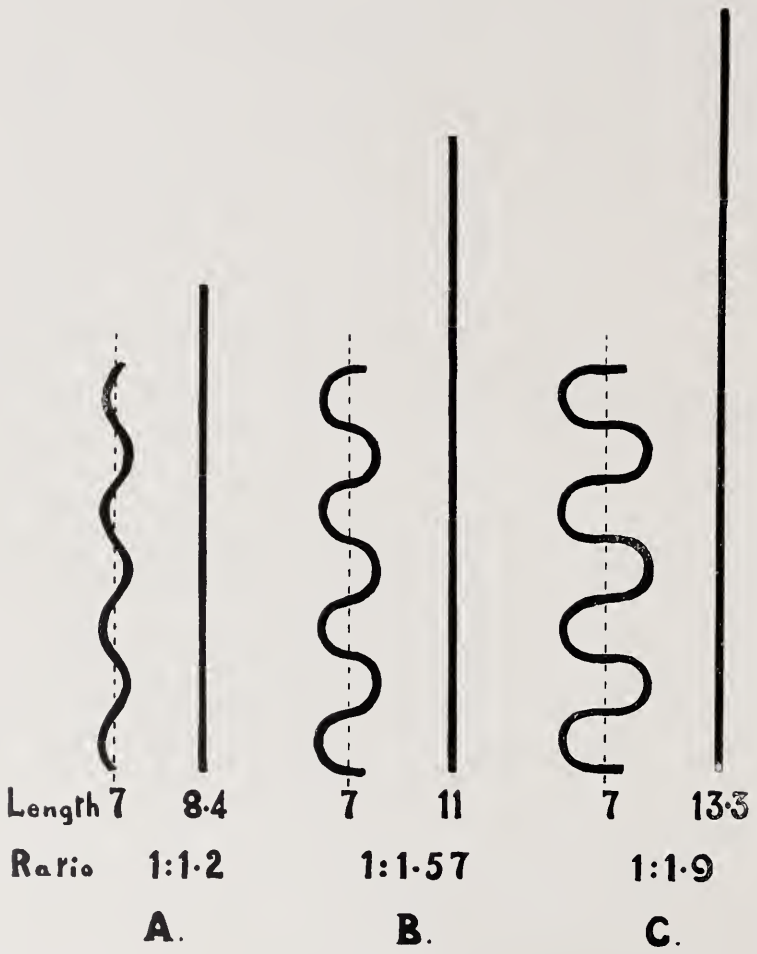

FIGURE 1.

The three main types of fibre crimping in Merino wool compared with their straight length. A, Shallow crimping.—The crimped length and straight length have a ratio of 1 : 1.2. *B, Half-circle crimping.—The ratio of the crimped length to the straight length is* 1 : 1.57. *C, Deep crimping.—Ratio* 1 : 1.9.

TABLE 2.

Crimped length and straight length of 20 fibres from a single staple of a well-defined, regularly crimped wool, showing differences in the ratio of the individual fibres, which are determined by differences in the form of the crimps. Average ratio 1 : 1.57.

Crimped Length.	Straight Length.	Ratio.	Crimped Length.	Straight Length.	Ratio.
68	101	1 : 1·48	80	138	1 : 1·72
78	117	1 : 1·50	87	132	1 : 1·51
78	112	1 : 1·43	81	131	1 : 1·61
85	139	1 : 1·63	84	111	1 : 1·32
85	134	1 : 1·57	79	112	1 : 1·41
80	112	1 : 1·40	76	111	1 : 1·46
82	129	1 : 1·57	82	127	1 : 1·55
93	127	1 : 1·36	95	158	1 : 1·66
78	113	1 : 1·45	83	132	1 : 1·59
84	128	1 : 1·52	75	102	1 : 1·36

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Quality Variation and Distribution in the Fleece of the Merino.

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1. Introduction.

The fleece of the Merino covers the whole body of the sheep, and extends partly over the head and tail, and down the fore and hind limbs. The wool has not everywhere the same character, whether it be examined on the sheep's back or after shearing. Even on the same fleece some parts will be longer than others, some will be denser, some will be finer, and some will be more closely and evenly crimped.

For the production of fabrics of the highest grades a wool should be as uniform as possible, and the ideal condition would be one in which the fleece is the same all over. The sheep breeder needs to keep in mind that wool now enters into competition with the artificial fibre which has all the uniformity that could be desired. Uniform fleeces would render unnecessary the process of sorting in the mills and would produce a far more desirable fabric. Dr. S. G. Barker has recently remarked: "If the doctrine of uniformity of fibre be made the text of the producer, then the future of the wool industries is more likely to be assured."

While a fleece displays differences such as the above on ordinary handling, the microscope reveals still further variability. Variation in thickness exists among the many fibres making up a staple, and even a single fibre is not the same at different places along its length. A wool may, therefore, be studied from many aspects, all of which influence its textile value. To a large degree the experienced woolman is able to estimate the differences merely by handling, though without assigning numerical values. For accuracy in the comparison of wools laboratory studies are necessary; these raise our knowledge from an empirical to a scientific basis.

Raw wools and tops are classified under their quality number, which has reference to fibre thickness. But the many other characteristics are also taken into account in estimating their value and the use to which they can be applied. The present study is limited to the various wool qualities making up the single fleece, and the way they are distributed over the sheep as a whole. It is part of a fuller study concerned with uniformity of wool production generally.

2. Quality and Quality Number.

A distinction needs to be made between quality and quality number. *Quality* is a descriptive term, and refers to the appearance and handle of a wool. It expresses the general impression which one first receives of a sample, and comprises various attributes recognisable on a ready examination by eye and hand. In its highest expression in the Merino quality refers to a wool with a well-defined and even crimp, a softness and pliability of feel, and a brightness and richness of appearance, a wool usually with a good colour and a rather fluid yolk. It is a general descriptive term, much better understood in practice than defined, and inclusive of many factors which are only measurable when separately analysed. It is probable that quality originally meant softness and pliability of handle, but as these are generally associated with even crimping and a bright appearance the latter have come to be included in the term. Qualifications, such as " high," " good," " low " and " poor," are added to denote differences of degree of quality. The attributes of length, soundness and uniformity are not comprised under quality, and the term may be applied equally to a fine, medium or strong wool.

Quality will have a different expression when used for wools of different breeds of sheep. Thus a high quality Blackface wool will not be the same as a high quality Merino, nor a high quality Lincoln, Romney or Cheviot.

Quality Number is a classificatory term, and has a more restricted usage than the word quality when used alone, and can be better defined. It is used in buying, selling, classing and sorting wools, and then has reference to the mean fibre thickness as indicated by the number of crimps. For the Merino the quality numbers range from 60's to 90's and upwards, those below 60's being conventionally classed as cross-breds, and those above 90's being rarely considered. Originally quality number was held to represent the spinning value or spinning count of a wool, and at times is still so regarded. But spinning value is found to depend upon so many factors, which are

scarcely determinable on a ready examination, that probably few quality number estimations are in agreement with the actual spinning power. Hence the term quality number has largely lost its primary significance and become restricted to denote fibre thickness. In practice the word quality alone, with "number" omitted, is generally used, but the classificatory sense is always clear and distinguishable from quality as used in the descriptive sense.

While then quality includes the handle and feel of a wool, its appearance and the character of the crimping, quality number is concerned with the number of crimps per inch as an indication of fibre thickness. In its descriptive sense quality takes no direct account of fibre thickness, while in a classificatory sense quality makes this the chief consideration. Quality is employed in the latter sense in all buying, selling, classing and sorting of wools.

The above is the usual significance of the terms as employed in South Africa, and is in conformity with Bradford usage. Thus in an article appearing in "The Wool Record," 24th October, 1929, the writer, "Tex," remarks: "The most important use of the term quality is in relation to the diameter of fibres. Wool sorting is based on fineness of fibre, and this is considered to be the soundest basis on which wools and top qualities can be classified. The top-maker, in fixing a quality number for his product considers fineness of fibre without regard to length, surface structure or soundness. A 64's quality wool is 64's whether the fibres are 4 in., 3 in., or 2 in. in average length, notwithstanding that there will be a considerable difference in the spinning property of the three classes. These properties, however, may be distinguished from one another by terming the three sections warp, weft or hosiery varieties of 64's respectively, thus indicating the differences in their spinning properties. Spinning property is a combination of fineness of fibre and adequate length of fibre to spin to the counts required, therefore, although the 2 in. length material is up to 64's quality, its shortness reduces its spinning property to considerably below that of the 64's which is 4 in. in length."

The writer likewise points out the clear distinction which must be made between the quality number of raw wools and tops and the count number of the yarns to which they can be spun. Strictly, quality number refers only to fibre thickness, while count number or spinning count represents the length of yarn which can be spun from a definite weight of scoured wool, and takes no direct account of the thickness of the fibre, and of the many other characteristics which influence the fineness to which a wool can be spun. From this it follows that the quality numbers given to raw wools in South Africa "whilst having some relationship to actual spinning value do not, and cannot, indicate exactly the length of yarn per pound to be obtained."

Originally quality number and count number had the same meaning, and in estimating the quality the woolman was understood to estimate the spinning value also. The latter is now known to be dependent upon so many factors, structural and dimensional, and often microscopical, that obviously they could not all be assessed in combination, even by the most experienced woolman.

In actual handling the quality number of raw wools does not admit of very precise determination, and any estimation has only a general application. Further, a sample estimated by a woolman as representing a certain quality will on close examination usually show two or three other qualities. Even different strands of a single staple will often include several qualities, while a closer analysis with the microscope will reveal fibres which, if grouped separately, would give wools of fine, medium and strong quality. Whatever its type no wool is made up wholly of fine fibres, or of medium, or of strong, but is a mixture of a large number of each kind; fine fibres, and more of them, however, occur in fine wools, and stronger fibres and more of them in strong wools. Fine wools have, however, a narrower thickness range than medium, and medium wools a less thickness range than strong wools (Duerden and Bosman, 1925).

On close analysis, therefore, all wools are fibre complexes, and the quality assigned them represents the average or mean thickness of the many components. From a large sample a trained woolman can select staples which conform to the standard for a particular quality, but they are usually intermingled with other qualities, and the sample as a whole is given a quality based on the average.

This is as far as the woolman or sorter can carry his analysis of a wool, and it is usually considered to be all that is necessary in practice, since in the main the method leads to the production of uniformly consistent tops and yarns. To gain a more intimate knowledge of their wools many firms, however, now resort to laboratory analysis.

3. Estimation of the Quality of a Wool.

For a true estimation of the quality number of a wool it is necessary to know the average thickness of the fibres. The actual fibres are, however, too fine to be measured in ordinary handling, and only an approximate estimation can be made by this means; an arithmetical determination calls for the assistance of the microscope and other appliances only available in a laboratory. Fortunately, it has been found that the crimps or waves, always present in Merino wool, may be taken as a fairly reliable guide to the thickness of a wool, and in practice these are relied upon as an approximate and ready means of estimating wool qualities.

Much study has been given to the nature and formation of the crimps (Barker, 1929), and particularly to the question of how far they can be accepted as a guide to the mean thickness of the fibres, in other words, as to how far they may be taken to represent the quality number (Duerden, 1929). It is highly desirable to know to what extent the woolman is justified in basing his thickness and quality estimates upon the number and the nature of the crimps. In the past different investigators have come to different conclusions, some finding no correlation between the number of crimps and fibre thickness, while others obtain a definite relationship. Much depends upon the selection of the material for study, and when proper care is exercised it is found that for the Merino a constant relationship can be demonstrated in a high proportion of cases.

Large numbers of samples, specially selected by experienced woolmen and with their quality numbers given them, have been procured, and the component fibres of type staples and strands

measured in various ways, after counting the number of crimps per inch. By this means it has been possible to ascertain and fix the thickness limits of each wool quality, and then to show that each corresponds with definite crimp limits.

From the study a Table of Standards has been compiled, giving side by side all the quality numbers of Merino wool, their thickness limits and their crimp range per inch. The qualities compared extend from 56's to 150's, but, as already remarked, in practice numbers below 60's are classed as cross-breds while quantities above 90's are rarely considered. The table appears below: —

TABLE 1.

Standard Thickness Limits of the Different Qualities of South African Merino Wool and their Range of Crimps per inch.

Quality Number.	Thickness Limits.	Crimps per Inch.
150's	14·0 — 14·7u	28 — 30
120's	14·7 — 15·4u	25 — 27
100's	15·4 — 16·2u	22 — 24
90's	16·2 — 17·0u	20 — 21
80's	17·0 — 17·9u	18 — 19
70's	17·9 — 18·9u	16 — 17
66's	18·9 — 20·0u	14 — 15
64's	20·0 — 21·3u	12 — 13
60's	21·3 — 23·0u	10 — 11
58's	23·0 — 25·5u	8 — 9
56's	25·5 — 29·0u	6 — 7

MM. Dantzer et Rœhrich (1929) have since made a thorough study of the thickness measurements of the complete range of quality numbers as accepted by French woolmen and compared the quality numbers with those in use in Britain and the Dominions. These are given in Table 2 below for the qualities 100's to 56's, while the mean theoretical thickness values ascertained for the corresponding qualities in South Africa are added in the last column. The two show a very close agreement.

TABLE 2.

Comparison of French and English Quality Numbers of Wool. The mean thickness of the French Qualities as determined by MM. Dantzer et Rœhrich are given and also those for South African Wools. The 66's quality adopted in South Africa is not included in the French Range.

French Qualities.	English Qualities.	Mean Thickness, French.	Mean Thickness, South Africa.
150's	100's	15·55u	15·80u
140's	90's	16·70u	16·60u
130's	80's	17·95u	17·45u
120's	70's	19·25u	18·40u
—	66's	—	19·45u
110's	64's	20·65u	20·65u
100's	60's	22·15u	22·15u
P's	58's	23·75u	24·25u
I's	56's	25·55u	27·25u

The results with South African wools have been incorporated in a Crimp Scale in which the number of teeth represents the number of crimps per inch, and the corresponding quality numbers have been added. Large numbers of the scale have been distributed to sheep farmers throughout the Union, who are now in a position to determine approximately the quality number of thickness of the wool they are producing.

The Scale has been employed on large numbers of selected wool samples and wool-matchings, procured both within the Union and from overseas, and the results have been checked from time to time by actual measurements. The agreements are such as to establish the reliability of the scale for the general determination of qualities. Complete agreement between crimps and fibre thickness is obtained in about 75 per cent. of cases, while the exceptions differ by not more than one quality. Fold wools and impoverished wools are often irregular as regards the relationship of their crimp and fibre thickness, and the scale is not recommended as a certain guide to these. The scale enables the quality of the wool to be determined with an accuracy equal to that which is possible to the farmer, woolman and sorter, which is all that is necessary for practical purposes.

4. Method of Study.

The definite values in crimps and fibre thickness now assigned to each wool quality, and the provision of a ready means for determining them, have made possible a practicable method of study of the various qualities represented in a fleece, and their distribution over the body. Previous efforts in this direction have been made by Hawksworth (1920) and Barker (1924-1925),* but not on a comparative scale for a particular breed of sheep.

Ten fleeces have been examined and the results are displayed in the accompanying charts (Figs. 1 to 10). The series gives an indication as to the quality variability to be met with in the Merino fleece, and has much bearing upon the important question of uniformity in wool production generally. The study is introductory to more extended and systematic efforts where flocks can be dealt with as a whole.

The following method has been adopted. Numerous skins of sheep, with the fleece already well-grown, have been obtained from farmers in various parts of the Union. No special selection has been made, the object being to secure as representative a series as possible of plain-bodied flock sheep, all stud rams with marked neck folds being left aside. Each fleece was divided into right and left halves by a median dorsal chalk line and one side only studied, both sides being found at an early stage to be approximately similar. Staples about an inch apart were then marked off, and the number of crimps to the inch measured by means of the crimp scale, either *in situ* or after shearing the staple.

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* In the Textile Museum at the University of Leeds, Prof. A. F. Barker has exhibited a large series of prepared skins representing the many British Breeds of sheep, with the wool upon them marked so as to show the quality distribution.

The entire fleece was carefully worked over, each quality as ascertained being added to the chart, and the approximate area occupied by each then marked out by dotted lines. In practice it was found that no sharp line of separation could be drawn between one quality and another, all grading insensibly. Also in nearly all cases the passage is from one quality to the next, whether finer or stronger; no sudden changes of more than a single quality are found, apart from cases of fold wool.

It is fully understood that the method followed does not meet all the requirements of a scientific study of quality and quality distribution. To do this would involve so much labour as to render it inapplicable in practice. It is to meet the general requirements of the breeder and woolman, though previous studies have indicated the limited value to be attached to the crimps as indicators of quality. Further, recent studies on the form and nature of the crimps have shown that these have a bearing on the desirability or otherwise of a wool from a breeder's point of view, quite apart from the number per inch or the quality indicated.

5. Description of Fleeces.

The fleece represented in Fig. 1 gives most nearly the fundamental pattern of the various qualities, and is almost schematic in its simplicity. It may be taken as typical of the Merino generally. The finest quality, 80's, occurs in the middle, largely covering the shoulders, sides and back. This is surrounded by the next finest quality, 70's, which extends forwards from the shoulder along the sides of the neck, and downwards over the belly, and then crosses from side to side over the hind part of the back. In front a tongue-shaped area over the back of the neck is covered with the next strongest quality, 66's, while behind also the 70's passes into 66's, this in turn merging into 64's.

The 70's, 66's and 64's form three transverse bands over the hind region, while the 64's extends down the hind legs, the covering of the fore legs being slightly finer, 66's. The under side of the neck has two small patches, one of 66's and the other of 80's. The wool here, however, may be influenced by the presence of the frontal folds, and in the neck not much reliance is ever placed upon the crimps as indicators of quality.

The quality distribution represented in Fig. 1 may now be compared with that in the other fleeces. The arrangement in Fig. 2 is from a fleece of somewhat stronger wool, and is not very different from that in Fig. 1. The two highest qualities, 70's and 66's, have practically the same relationship as before, the finest being in the middle and surrounded by the next finest. The neck-tongue, 60's, is the strongest quality in front, and the under neck has no special patches, while the rump and britch are interrupted in the middle by a coarse tail patch of 58's. The covering of the front part of the hind leg is slightly finer, 64's, than that of the hind part and britch, 60's.

The fleece represented in Fig. 3 is unusual in the small area covered by the finest wool, 66's, which is encroached upon along the back by the next finest, 64's. The finest wool, 66's, occupies an area

along each side, while the middle of the shoulders and back, which is usually covered with the finest, now shows the second quality, 64's. The neck-tongue, 60's, is well-defined, but no special patches cover the under neck. The rump band, 60's, is wavy and passes down the hind legs The britch band is broken up into three separate patches of 58's.

The fleece shown in Fig. 4 is very largely made up of the highest quality, 70's, the second quality, 66's, forming the margins in front and behind, and constituting the neck-tongue and rump band. The 80's covering the belly is unusual, but this region is well known to produce wools which may be out of harmony with the rest of the fleece. While the crimps indicate a wool of 80's, actual measurement of the fibres shows the wool to be of a lower count.

In Fig. 5 the two highest qualities, 66's and 64's, are intermingled and distributed over nearly the entire body. The neck-tongue, fore-limb and rump band are 60's, while the britch band is broken up into the two patches of the coarsest, 58's. The finest wool does not occur over the shoulder region, as is usually the case.

The fleece in Fig. 6 shows also considerable mingling of the two highest qualities, 80's and 70's, the coarser, 70's, being more central and partly crossing the shoulder area and the middle of the back. The third quality, 66's, is unusually well developed, while the rump band and britch band are typical.

In the fleece shown in Fig. 7 the two highest qualities, 66's, and 64's, occupy a comparatively small proportion of the entire fleece and, as in Fig. 6, the stronger of the two, 64's, is surrounded by the finer, that is, the shoulder and back areas are stronger than the greater part of the sides. The other qualities conform with the general plan, the britch wool, 58's, forming an unusally wide band.

Fig. 8 represents a very uniform fleece, with only a small area in the middle covered with the finest quality, 70's, and a much larger area with the second quality, 66's. The two hind bands, 64's and 60's, are much as usual, and a small neck patch of 70's is present.

In Fig. 9, two patches of the third quality, 66's, are included within the second quality, 70's, the rest of the 66's surrounding the 70's. The small single patch of 80's on each side above the axilla is not unusual, for frequently this area is found to produce a highly crimped wool. The britch band is wider than that of the rump.

In Fig. 10 the two highest qualities, 70's and 66's, constitute a great proportion of the fleece, the finest, 70's, as in Fig. 8, occurring as an island surrounded by the next finest, 66's; here, however, the greatest length of the island is across the shoulder area, while in Fig. 8 it is more along the back. The neck-tongue, 64's, is very reduced compared with that in the other fleeces.

6. Range of Quality Variation.

The ten charts reveal that in most instances a single fleece ranges over four successive qualities. In some cases five different qualities may be present, as in Fig. 6, but the one extreme is represented by only two small patches of coarse wool, 60's, which have little or no bearing upon the general character of the fleece. In no instance does a sheep produce less than three or four different qualities.

The result has much practical significance. It indicates to the farmer, woolman and sorter the degree of variation which he may expect to find in wool belonging to a single fleece. Only four, or possibly five, qualities will ever occur in any quantity, and they will merge into one another; no quality very divergent from the general bulk will be found.

Intermingled with the finest quality may be small staples of still finer wool. Thus along with an 80's may be a small amount of 90's or 100's, but these are only of importance when in sufficient quantity to justify sorting out. Similarly mixed with the coarser qualities may be staples of still lower quality. In other words, the qualities are by no means as sharply defined as is generally supposed, and from any sample of one quality small quantities of other qualities may be picked out.

A fleece fine in one part would in general not be coarse in another; and a coarse fleece would include no very fine wool. If an entire clip were from a fairly uniform flock then the extent of range of the qualities could be estimated from a representative sample, and would be correct within fairly definite limits. Any great divergence of quality in a clip would signify a mixed flock. The general quality average of a fleece or a clip will determine whether it will be classed as fine, medium or strong.

The proportion in which any one quality occurs is also very variable. A single quality may make up almost the entire fleece, as in Fig. 8, or the different qualities may be present in nearly the same proportions, as in Fig. 6. As will be shown later, however, the two highest qualities are usually the dominant wool, the two lowest qualities occurring in much smaller proportions.

7. Distribution of the Various Qualities on the Sheep.

At first the various qualities of wool seem to be arranged on the sheep in a haphazard manner, as if any quality might appear at any place, and no two sheep are alike. But comparison of the ten fleeces shows that they can be reduced to a fairly definite plan, which is applicable to the Merino fleece generally. It is known that in a general way the finest wool of the fleece occurs over the shoulders and along the back and sides, while the strongest is found towards the hind region over the rump and britch, and in front over the neck folds. A similar relationship holds in most mammals with a long coat, and has been noticed by other writers. Thus the coat is longest in front and behind in the lion, camel, musk-ox, yak, collie dog and others, often forming a mane in front and a less development over the hind legs. It seems likely that this strong development of hair at the two extremes, in front and behind, is associated with protection against exposure to cold winds and rains, an animal usually placing itself in the direct course of the wind.

In all the fleeces examined three groupings of the qualities can be distinguished: (*a*) *The dominant or bulk wool* over the body generally; (*b*) *The neck wool*; and (*c*) *The rump and britch wool.*

Everywhere it will be found that a fleece is largely made up of its two highest qualities, which for most purposes may be regarded as one. They may be termed the *dominant or bulk wool*, and where necessary distinguished as *first* and *second dominants*. In all the

fleeces the dominants constitute by far the greater part of the covering, and are to be found over the sides of the neck and continued over the shoulders and along the back and sides to the hind region, stopping short over the rump, and usually including the belly wool.

The two highest qualities are not often found as regularly arranged as in the fleece represented in Fig. 1, but may be intermingled and replace one another in varying degrees. Usually the finest quality will be about the middle of the fleece, and the sides will represent the next finest, but occasionally, as in Figs. 3 and 6, the second finest will be in the middle, and will be bordered by the first finest. While, therefore, the highest quality wool will usually be found around the shoulder, the next quality will sometimes occupy this area; one may merge into the other at almost any part. *Taking the fleece as a whole the first and second qualities constitute the dominant wool, and may be regarded as interchangeable as far as concerns their relative positions on the sheep and the proportional area covered by each.*

The first and second dominants can be easily separated on a careful examination of a fleece, yet in the ordinary practice of wool classing and wool sorting this is rarely resorted to, and the two are taken together. At these stages an average or mean quality estimate is all that is desired, and will usually be decided according to which of the two dominants is present in greater quantity. As already shown, most commercial wools, however well selected, tend to include staples or strands representing three or four qualities. An ordinary quality estimate nearly always represents the average or mean of several qualities.

Since the two dominant wools comprise most of the fleece they will determine whether the wool as a whole will be classed as fine, medium or strong. A fine Merino wool usually includes all qualities ranging from 70's upwards, a medium wool qualities from 64's to 66's, and a strong wool 60's. If only two classes are made then the fine wool will generally include all above 64's, and the strong wool 64's and below.

Neck Wool.—The neck wool is usually very variable in quality, especially at the sides and beneath, in association with the presence of the neck folds or pleats. These are nearly always present to some degree, even in what appear to be plain-bodied sheep. The crimping along the edge of the folds tends to differ much from that in the depression between the folds, and the true thickness can be ascertained only by actual measurement of the fibres. Sometimes patches will occur stronger than the dominant wool, and sometimes finer; where pleats occur along the sides of the body the wool grown over them is also of the same irregular character, and interferes with the uniformity of the dominant wool. This is well seen in the strip of skin represented in Fig. 11.

The upper surface of the neck appears to be always covered by wool which is stronger than the two dominant qualities, and is arranged to form a tongue-shaped patch which may be called the *neck-tongue*.

Rump and Britch Bands.—The rump and britch wools constitute a third grouping of qualities, and are always the coarsest part of the fleece, that over the britch being usually a quality stronger than that over the rump. Generally both occur in the form of transverse bands extending across the back and sides of the sheep, the britch quality usually passing down the hind limbs. Sometimes the last band will be interrupted in the middle, either by the finer rump or the stronger tail wool.

In the study no account has been taken of kemp and gare, since the fibres do not occur in any of the fleeces examined. These coarse fibres have been shown to belong to a category altogether apart from wool fibres, and their thickness measurements do not grade into those of wool (Bliss, 1926).

8. Discussion.

From the foregoing it is manifest that only in plain-bodied sheep will the qualities occur with the uniformity represented in the charts. Where the skin is wrinkly, the wool growing over the folds will show a different crimping from that over the body generally, and would be classed as a different quality. This is particularly the case with the neck folds of rams, over which three or four qualities may be represented, but it applies also to the slight folds or pleats occurring over the sides of the sheep, and not necessarily showing as wrinkles at the surface. On opening up the fleece over the sides a few staples of wool much coarser than the rest will often be disclosed, and close examination will show they are growing on a narrow pleat (Fig. 11). On account of the folds the neck wool at the sides and under is also generally patchy and irregular in its crimping, and is an admixture of different qualities, sometimes removed in careful classing.

Despite these apparent divergences the comparative study shows that the quality distribution of the Merino fleece is reducible to a common plan. The general body of the sheep will be covered by the two highest qualities, the dominant or bulk wool, either separate or intermingled; the neck region will be stronger than these two, and at least two stronger qualities will occur behind.

The survey of all the fleeces indicates that the one represented in Fig. 1 most nearly conforms to what may be regarded as this fundamental plan. It shows that in general the highest quality, the finest fibres, will be found about the middle of the fleece, covering the shoulders, sides and back. This will be surrounded by the next finest quality, which will extend forwards from the shoulder over the sides of the neck and downwards over the belly, and pass over the hind region of the back. A still coarser quality will cover the upper part of the neck, and irregular patches may appear over the sides and under part of the neck. A coarse zone will be found covering the rump, and the coarsest wool of all will occur over the britch and around the root of the tail.

9. Production of a Uniform Fleece.

The arrangement of the wool qualities roughly into those of the head and neck region, the shoulder, back and side region, and the hind region inclusive of the limbs, may be taken to represent the natural grouping of the fibres, one characteristic of the sheep apart

from any selection. The shoulder area is that of finest wool production, and from this it coarsens in all directions, more rapidly towards the head and more gradually backwards. As already seen, other attributes being equal, the more uniform is a wool in quality the greater is its desirability as a textile material; and the question may well be asked, can the sheep be made to produce a fleece of more uniform quality?

In every case it would be the dominant or bulk wool, the two highest qualities, which would call for extension, to replace the stronger head and neck wool along with that of the rump and britch. With the present insistence on finer wool by the buyer it is hardly conceivable that any breeder will strive to increase the area covered by the stronger wools at the expense of the finer.

The charts show that the two dominant wools vary much in the extent to which they constitute the whole fleece; sometimes they cover almost the whole body; and there can be little doubt that if consistent breeding were adopted they could be made almost the exclusive covering. If a character or condition is present in one or more individuals of a flock it is justifiable to assume that by selection in breeding it could be intensified and made to appear in all the members; and within Merino flocks are to be found individuals in which the desired uniformity occurs. In all probability a greater uniformity among the individual fibres would also result from a uniform quality distribution.

10. Summary.

1. The paper gives an account of the quality or thickness variations in individual fleeces of the Merino, as well as their distribution on the sheep, and attempts to reduce the variations to a common plan. It is part of an effort concerned with uniformity of wool production generally.

2. The term quality is used with two different meanings. As a descriptive term it refers to the appearance and handle of a wool, its softness and pliability, and the nature of the crimps, while as a classificatory term, "quality number," it refers to the number of crimps per inch which are found to be closely correlated with the mean or average thickness of the fibres. Quality is employed in the latter sense in buying, selling, classing and sorting wool.

3. Quality number is also to be distinguished from spinning number or spinning count. The former refers to the mean or average thickness of the fibres, while the latter refers to the spinning value of the wool, and includes the quality number or thickness as a factor, as well as all the many other attributes of a wool which influence its spinning.

4. A Table of Standards gives the range of limits of the crimps for each quality number and also the thickness range. A cardboard Crimp Scale has been designed and distributed among farmers, who are now in a position to make general estimates of the quality or thickness of the wool they are producing.

5. The various qualities, based on the number of crimps, occurring on ten different plain-bodied fleeces have been traced, as well as their distribution, and are represented in the form of diagrams.

6. It is shown that in the fleece the passage is gradual from one quality to the next, whether finer or stronger, and no sharp line separates one quality from that adjacent. Often one quality will be intermingled with another, but no marked sudden changes occur, apart from those in fold and pleat wool, where the crimps are no certain guide to quality.

7. A single fleece usually ranges over four successive qualities, rarely five. A fleece fine in one part will not be coarse in another, and a coarse fleece will include no very fine wool. This does not apply to sheep with gare or kemp; wool and kemp are two wholly distinct types of fibres, though intermingled with one another.

8. The qualities are different for different fleeces, and it is the general average of the qualities which determines whether a fleece will be classed as fine, medium or strong; a fleece averaging 60's is considered as strong, one of 64's and 66's as medium, and one of 70's and upwards as fine.

9. The proportion in which any one quality appears in a fleece is variable, but the two highest qualities taken together are usually far in excess of the others, while the two lowest qualities occur in small proportions.

10. In general three groupings of the qualities can be distinguished on a fleece: the *dominant or bulk wool* over the body generally, representing the two highest qualities; the *neck wool*; and the *rump or britch wool*.

11. Comparison is made of the quality distribution in ten different fleeces. While apparently very diversified, the distribution is shown to be referable to a common plan. The general body of the sheep is covered with the two highest qualities, the dominant or bulk wool, either distinct from one another or intermingled; the upper surface of the neck will be stronger than these two, while at least two lower qualities will occur over the rump and britch areas.

12. If consistent breeding were carried out, fleeces from a single flock would be expected to show greater uniformity in quality distribution than a haphazard selection from different flocks, particularly if the farming conditions were uniform.

13. The question is discussed as to whether the fleece of the Merino can be changed so as to produce a wool of more uniform quality, a condition which is much to be desired, especially in view of the uniformity of the competing artificial fibre.

14. Individual sheep are to be found in which nearly the whole body is covered with wool of the dominant quality, hence it is held that by consistent breeding it would be possible to extend this over the rest of the sheep and include the flock generally, and so increase the uniformity.

15. A high degree of uniformity in quality over the sheep would doubtless be accompanied by less variability in the individual fibres of a staple.

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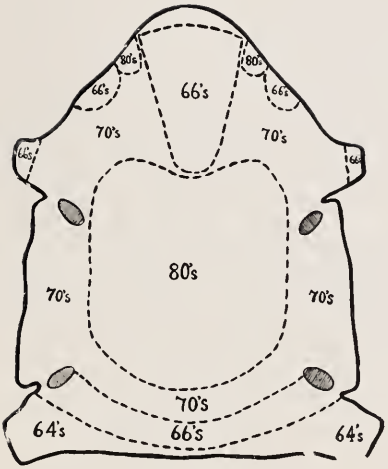

Fig. 1.

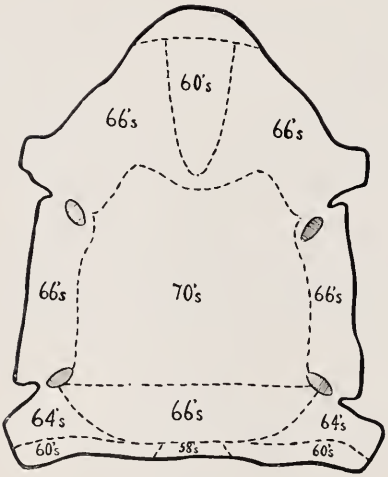

Fig. 2.

Fig. 1.—Figs. 1 to 10 are plans of distribution of the various wool qualities in ten different plain-bodied Merino flock fleeces. The observations were made on dried skins with the wool attached, and the outline of the skin is represented by the thick boundary of each figure. The two shaded patches in front represent the axillary bare patches and the two behind the inguinal patches.

In Fig. 1 the two highest qualities or dominant wools, 70's and 80's, occupy almost the whole fleece, the two lowest qualities, 64's and 66's, covering small areas towards the margins or "points."

Fig. 2.—The two dominant wools, 70's and 66's, again constitute almost the entire fleece, small patches varying from 64's to 58's occurring behind. In this, as in most of the fleeces, the neck-tongue is well-defined, and represents a slightly coarser wool, 60's.

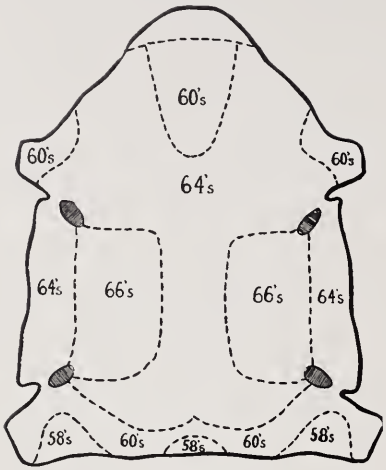

Fig. 3.

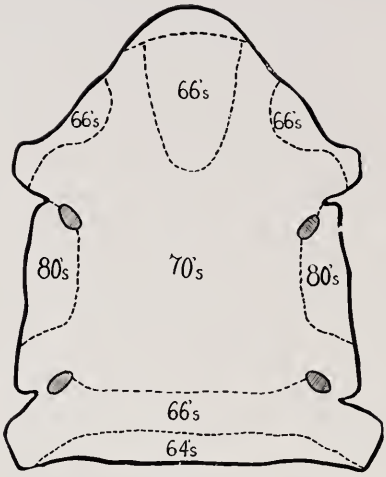

Fig. 4.

Fig. 3.—The finest wool 66's, occupies an area along each side, while the middle of the shoulders and back, which is usually covered with the highest quality, now shows the second quality, 64's.

Fig. 4.—The greater part of the fleece is covered with 70's quality, and is largely surrounded by the next quality, 66's. The crimping of the belly wool indicates an 80's, but actual fibre measurement shows the wool to be of a coarser quality.

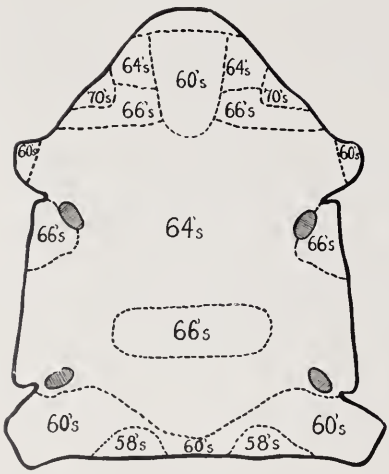

Fig. 5.

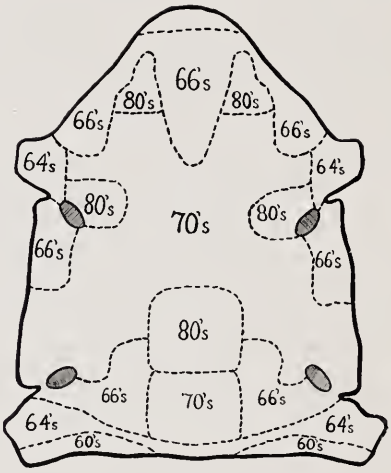

Fig. 6.

Fig. 5.—The two dominant qualities, 64's and 66's, are much intermingled, and cover almost the entire skin, merging behind into 60's and 58's, with a small patch of 70's in the neck region.

Fig. 6.—The two highest qualities, 70's and 80's, are much intermingled and the third quality, 66's, is well developed, passing down the legs and behind into the coarser 64's and 60's.

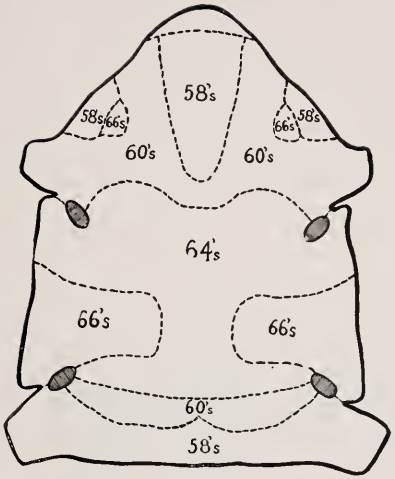

Fig. 7.

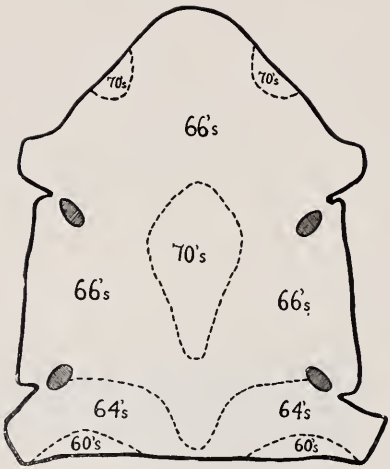

Fig. 8.

Fig. 7.—The two highest qualities, 64's and 66's, occupy a rather small proportion of the entire fleece, a considerable intermingling of all the qualities occurring.

Fig. 8.—The fleece is here more uniform than usual, a 66's quality covering the greater part of the area and surrounding a patch of 70's in the middle.

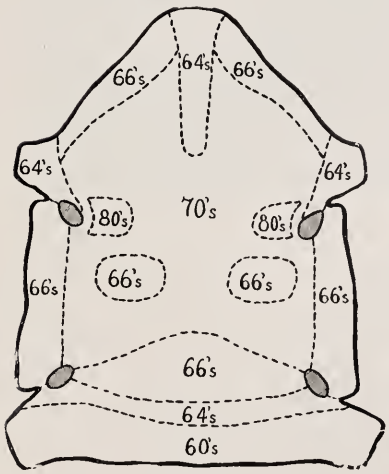

Fig. 9.

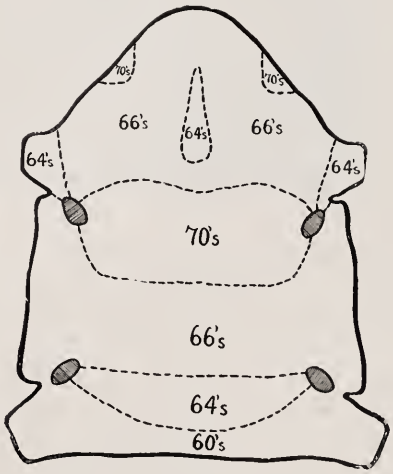

Fig. 10.

Fig. 9.—The various qualities are here intermingled to an unusual degree, and two patches of the third quality, 66's, are included within the second quality, 70's. Above each axilla is a single patch of the highest quality, 80's. Wool finer than elsewhere is occasionally found in this position.

Fig. 10.—The two dominants, 66's and 70's, constitute a great proportion of the fleece, the finest being surrounded by the next quality. The neck-tongue, 64's, is very reduced.

Fig. 11.

FIG. 11.—Photograph of strip of skin from the side of the body, showing projecting pleats, the wool over which is crimped differently from that growing between the pleats.

17th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1931.

Rates of Growth of Merino Wool Month by Month.

By Dr. J. E. DUERDEN, Director of Wool Research, and G. S. MARÉ, B.Sc.(Agr.), Sheep and Wool Research Officer.

The fleece of the Merino grows in length month by month without any shedding, and from year to year if shearing does not take place.* The growth is continuous, but rarely uniform in rate, varying in length as well as in thickness according to the state of nutrition of the sheep. Various experiments are in progress to determine the nature of these variations and the conditions under which they occur. It is sometimes stated that the earlier growth after shearing is more rapid than the later; that is, that two shearings of six months wool give a heavier return than one of twelve months; but more data are necessary to prove or disprove this.

An experiment has been concluded at the Grootfontein School of Agriculture in which the coat of a lamb (No. MBA 3800) has been allowed to continue its growth uninterruptedly for thirty months without being shorn. The fleece had then a staple length of 7½ inches and the fibres an average straight length of nearly 13 inches. Clippings from the shoulder region were taken at approximately monthly intervals (Fig. 1), and length measurements made of the samples. The results are shown in Table 1.

* A sample of Merino wool, 70's, obtained from Mr. H. J. Harraway, Port Elizabeth, is 10 inches in staple length, and of uniform growth throughout; the separate fibres having a straight length of 14.6 inches. It probably represents a continuous growth of at least three years.

Hawkesworth in "Australasian Sheep and Wool", Sydney, 1920, p. 291, remarks: "Some time ago, for experiment, several Merino sheep were left unshorn for ten years. The wool at the end of this time was about 12 inches long. The growth of the first year was 2½ inches; second, 2 inches." Again, on p. 436, he states: "The sheep, as already mentioned, is an animal which does not, like most other animals, change its covering with the season. Its wool grows without hindrance, unless disease causes such, when it loses its previous growth. The successive yearly growth of wool is not proportionate if left unshorn. It rather decreases gradually, insomuch that wool, which has been left growing several years uninterruptedly, shows a yearly decrease in length. If the growth of the wool reaches the first year, a length, say, of 3 inches, in the second it will be about 2½ inches, in the third 2 inches; so that in samples of 8 years' successive growth, the last year's will attain the length of a little over ½ an inch." These results are altogether at variance with those of the present experiment.

Table 1.

Rate of Growth of Merino Wool, month by month, for a period of 29.5 Months.

Clipping No.	Date of Clipping.	Days of Growth.	Average Straight Length of 25 Fibres.		Growth between Clippings in cm.	Average Growth per day in cm.
			Inches.	Cm.		
Growth for Twelve Months, 28.5.27 to 30.5.28.						
1	28/5/27	Newborn	0·51	1·29	—	—
2	28/6/27	31	0·83	2·11	0·83	0·027
3	28/8/27	61	1·68	4·26	2·14	0·035
4	28/9/27	31	2·12	5·38	1·13	0·036
5	28/10/27	30	2·58	6·54	1·16	0·039
6	2/1/28	66	3·39	8·61	2·06	0·031
7	30/1/28	28	3·83	9·72	1·11	0·040
8	27/2/28	28	4·41	11·20	1·48	0·053
9	26/3/28	28	4·42	11·23	0·03	0·001
10	23/4/28	28	4·77	12·12	0·89	0·032
11	30/5/28	37	5·36	13·63	1·51	0·041
Growth for Twelve Months, 30.5.28 to 3.6.29.						
12	27/6/28	28	5·78	14·69	1·06	0·038
13	30/7/28	33	6·22	15·80	1·11	0·034
14	27/8/28	28	6·54	16·63	0·83	0·030
15	24/9/28	28	7·14	18·15	1·52	0·055
16	22/10/28	28	7·53	19·14	0·98	0·035
17	19/11/28	28	7·91	20·10	0·97	0·035
18	18/12/28	29	8·20	20·83	0·73	0·025
19	15/1/29	28	8·85	22·42	1·59	0·057
20	11/2/29	27	9·24	23·49	1·06	0·039
21	11/3/29	28	9·50	24·14	0·65	0·023
22	8/4/29	28	9·95	25·28	1·14	0·041
23	6/5/29	28	10·13	25·73	0·44	0·016
24	3/6/29	28	10·41	26·44	0·71	0·026
Growth for Five and a Half Months, 3.6.29 to 28.11.29.						
25	29/7/29	56	11·31	28·74	2·30	0·041
26	26/8/29	28	11·89	30·20	1·46	0·052
27	23/9/29	28	12·21	31·03	0·83	0·030
28	21/10/29	28	12·54	31·87	0·84	0·030
29	18/11/29	28	12·89	32·74	0·87	0·031

Summary.

Period.	Straight Length at End.	Growth during each Period.	Growth per Month.
	cm.	cm.	cm.
First twelve months.....	13·63	12·34	1·028
Second twelve months.....	26·44	12·81	1·068
Last period, 5·5 months.....	32·74	6·30	1·146

Average growth per month for entire period, 1·066 cm. or 0·419 inches.

The clippings are given consecutive numbers, and the date of clipping and days of growth represented by each are added, starting with the coat of the new-born lamb. The number of days between successive clippings is usually twenty-eight, but in the case of Nos. 3, 6, and 25, the growth represents a two months' interval.

The length of the clippings has been ascertained by drawing out from the cut end of the staple twenty-five separate fibres at random, and measuring each independently against the edge of a scale, the average of the measurements being given. In making the measurements the fibre is held straight by fine forceps at each end with the crimps eliminated, but without any stretching, when the length is necessarily somewhat more than it would be in its natural crimped state. The readings were taken in sixteenths of an inch, but are converted into inches in the fourth column and into centimetres in the fifth column. In the sixth column is given the length between the consecutive clippings, which represents the growth for the period, while in the last column is the average growth per day.

The straight length of the wool at the end of the first year is 13.63 cm. (5.36 inches), but as the average length of the fibres on the new-born lamb was already 1.29 cm. (0.51 inches), the actual growth during the twelve months was 12.34 cm. (4.85 inches), which gives an average of 1.028 cm. (0.4 or 2/5 inches) per month.

The length at the end of the second year is 2.644 cm. Taking from this the length for the first year we have 12.8 cm. as the actual growth during the second twelve months, in comparison with 12.34 cm. for the first year, a difference of only 0.47 cm. The average growth per month is 1.068 cm. in place of 1.028 cm. for the first year, the difference being so small as to have no significance.

At the end of the two and a half years the fibres have an average straight length of 32.74 cm., that is, 12.89 inches or over a foot. Taking from this the length attained during the first two years we obtain 6.30 cm. as the growth added during the last period, the average per month being 1.146 cm., which is practically the same as for the first and the second year.

The table shows that the actual increases as measured from the clippings are not equal month by month. The increase in Nos. 9, 21, and 23 is much smaller than the average, while in some of the others it is larger. The differences may be due to variations in the length

of the staples making up the samples; but they may also represent differences in the rate of growth during the particular periods, dependent upon nutritional variations. The average growth per month during each of the three preiods is, however, almost the same, namely, 1.066 cm., or 0.149 inches. It is doubtless of some significance that in each year the shortest growth is from March to May, that is, about the end of the summer period and the early winter.

The average monthly growth during each of the two complete years is thus practically the same, and follows the same rate during the short remaining period. *It is therefore highly suggestive that under ordinary conditions the average growth in length of the Merino fleece is the same from year to year, that is, the fleece continues to grow at about the same average rate whatever its length; a fleece during the first six months after shearing will grow at approximately the same rate as during the second six months and, if unshorn, the same length would be added during the second year as during the first; and so on.*

It is manifest that if a much longer Merino wool were desired for trade purposes it could be procured by allowing the sheep to remain unshorn, just as a shorter wool is obtained by shearing at less than twelve months; also, it may be expected that the length of wool would be proportionate to the length of time between shearings.

Prolonged exposure, however, leads to deterioration at the extremity of the fibre, and the tip breaks away in small successive fragments.* Hence it is not likely that the fleece would increase indefinitely in length, any more than in the case of human hair.

Divided into six-monthly periods the first half of each series will represent the second half of the calendar year and the second half of each series the first half of the year, that is, the first half will represent the growth during the end of winter and beginning of summer and the second half the end of summer and beginning of winter (southern hemisphere). The average growth in six-monthly periods is given below. A slight advantage appears in favour of the first period of each series, that is, the end of winter and early spring as compared with the summer and winter.

TABLE 2.

Average growths in six-monthly periods.

Winter and spring (1927)	6.29 cm.
Summer and winter (1928)	6.05 cm.
Winter and spring (1928)	6.47 cm.
Summer and winter (1929)	5.59 cm.
Winter and spring (1929)	6.30 cm.

Different sheep may be expected to give slightly different rates, strong wool growing more rapidly and longer than fine. The actual figures therefore apply only to the particular sheep with a fleece of quality number 70's, but for all Merinos an average uniformity of growth year by year may be expected.

* Duerden, J. E., and Boyd, E.: "The Blackface Persian—a Primitively-coated Fat-rumped Sheep." *Dept. of Agric. Bulletin* No. 82, 1930, p. 29.

Results from a single sheep may be regarded as of not more than suggestive value, and a second experiment with three male lambs is now being carried out.

Addendum.

Since the paper was submitted for publication a contribution on the same subject has appeared in the February number of *The Journal of the Textile Institute* (Vol XXII, 1931). The paper: "Monthly Wool Growth of Rambouillet Ewes," is by Robert H. Burns, University of Wyoming, U.S.A., at present carrying out investigations at the Animal Breeding Research Department, University of Edinburgh. The experiments on which it is based were continued for four years, using in each case six ewes of five different breeds of sheep. The results arrived at are in closest conformity with those here presented, and are summarized by the author as follows:

> "The monthly wool growth of Rambouillet ewes was remarkably uniform throughout the year, and in the four different years."
>
> "Wool growth during the first six months after shearing was only slightly greater (52 per cent.) than during the following six months (48 per cent.)."

FIG. 1.—Staples selected at intervals of three months over a period of from 3 to 30 months.

The Effect of Barley in the Ration on the Quality of Bacon.

By D. J. SCHUTTE, B.S.A., M.Sc., and C. A. MURRAY, B.Sc.(Agric.),* Division of Veterinary Services and Animal Industry.

The results of previous experiments reported by Romyn and others (1930) showed that barley in the ration of bacon pigs resulted in increased firmness of the back-fat. Itwas further observed that in some cases this feedstuff appeared to influence the type of side produced, while in other trials no such effect was noticed. In the present trial, carried out at the School of Agriculture and Experiment Station, Potchefstroom, Transvaal, the proportion of barley was increased in order to obtain further evidence on the possible effect of this feed on length of side and texture of fat produced.

Plan of Experiment and Experimental Methods.

Thirty-three Large White × Large Black cross-bred weaners were divided into three equal groups of eleven each, and fed in dry lot according to the following plan:—

Group I. Maize-meal, 90; meat-meal, 10.
Group II. Maize-meal, 45; barley-meal, 45; meat-meal, 10.
Group III. Maize-meal, 20; barley-meal, 70; meat-meal, 10.
(Proportions by weight.)

All groups had free access to a mineral mixture consisting of bone-meal, 3 parts and salt, 1 part, and were given a small amount of green feed in equal quantities daily, besides a regular supply of fresh drinking water.

The concentrates were fed twice daily in the form of a thick slop, the quantity of feed offered at one feeding being gauged by the amount the animals consumed in 15 to 20 minutes' time.

The pigs were weighed regularly at 14-day-intervals until they approached market weight when weekly weight records were kept. When finished, the animals were railed to the Farmers' Co-operative Bacon Factory at Estcourt, Natal, a distance of 400 miles, where they were killed, graded, and detailed data recorded on all carcases.

* Since resigned from the Department of Agriculture.

The methods employed in the dressing and grading of the carcases and in the determination of refractive indices were fully described by Romyn and others (1930) in the report referred to above.

Experimental Data.

Table I.

Live Weights, Daily Gains, and Average Feed Consumption.

	Lot I.	*Lot II.*	*Lot III.*
Number of pigs	11	11	11
Average intial age in days	72	70.4	71.5
Average final age	253.1	217.0	225.3
Average number of days in experiment	181.1	146.6	153.8
Average initial weight (lb.)	41.9	40.3	41.0
Average final weight	188.4	197.7	198.6
Average total gain in weight	146.5	155.6	157.6
Average daily gain in weight	0.81	1.06	1.03

Feed Consumption.

	Lot I.	*Lot II.*	*Lot III.*
Total concentrates consumed (lb.)	4,201	6,478	6,216
Average daily feed per pig	2.90	4.02	3.67
Feed consumed per 100 lb. live weight	385.5	301.0	285.0

Table II.

Weights, Measurements, and Grading of Carcases.

	Lot I.	*Lot II.*	*Lot III.*
Average weight at factory (lb.)	169.0	177.6	181.4
Average shrinkage in transit (per cent.)	10.3	9.9	9.0
Average dressed weight	143.5	144.6	146.8
Average dressing percentage	84.9	81.4	81.0
Average length of side (inches)	28.9	29.6	29.5
Average depth of side	15.80	16.10	16.00
Average depth of back-fat	1.55	1.60	1.49
Average circumference of ham	22.9	23.2	23.7
Average weight of cured side	49.8	51.0	51.0
Average percentage of $\frac{3}{4}$-side of full side	67.7	68.0	68.6
Texture of back-fat—			
Firm (per cent.)	37.50	72.70	63.60
Medium firm	37.50	17.30	36.40
Medium soft	25.00	10.00	—
Soft	—	—	—
Average refractive index	1.4599	1.4595	1.4594
Grading of Sides—			
No. 1 lean sizeable	87.50	63.60	63.60
No. 2 lean sizeable	—	9.10	18.20
No. 1 medium	22.50	27.30	18.20
No. 2 medium	—	—	—

Discussion of Results.

Table I indicates that the pigs made poor gains on all the rations, the results on the maize-meal–meat-meal ration being especially disappointing. The primary cause of the poor gains was put down to low-feed consumption. Ascaris infection and insufficient

exercise were contributing causes to the subnormal appetites. There is no significant difference in the gains in the barley-fed groups, both of which showed greater daily gains than the maize-fed group.

The pigs of Group I ate very sparingly, which accounts for the poor gains and the relatively large amount of concentrates required per unit of gain. The daily ration was barely sufficient for maintenance and normal growth. The pigs of Groups II and III showed better appetites and the amount of feed consumed per unit of gain can be considered very low in view of the slow rate of gain.

Referring to Table II, it is observed that the pigs in Group I showed a relatively high average dressing percentage, as compared with those in Groups II and III respectively; consequently, the average dressed weights were very similar for all groups, in spite of the fact that the pigs of Group I were killed at a much lower average weight than those of Group II.

The rations containing barley produced slightly longer pigs than the straight maize-meal–meat-meal ration. How much of this difference is due to the barley is difficult to determine, since it is quite probable that the lower killing weights of the Group I pigs may have influenced the length, although the greater age at which the pigs of this group were killed would tend to influence length in the opposite direction. It is interesting to note, however, that an appreciable increase in the proportion of barley in the ration had no additional effect upon length or grade. It should also be noted that the differences in length apparently had no effect on the grading of the carcases. Grade is determined by several factors, including degree of finish and length in relation to depth. Group II, which produced the highest percentage of sides of medium grade, showed the best finish and the greatest depth of side relative to length. The percentage of lean sizeable sides produced by all groups is very satisfactory. It would appear that low marketing weights, combined with relatively slow gains, especially in the case of Group I, influenced the grading of the sides, since a much lower percentage of No. 1 lean sizeable sides was obtained in all earlier trials in which this ration was employed.

In regard to texture of back-fat, it is evident that barley had a distinctly hardening influence. The average differences in refractive index between Group I and Groups II and III respectively represent more than a complete factory grade. It is significant, however, that the increase in barley from 45 per cent. in Group II to 70 per cent. in Group III had no correspondingly increased effect on texture.

The pigs of Group I produced a higher percentage of firm carcases than is usually produced on this ration. The relatively high marketing ages of the pigs would have some influence in this respect according to the results obtained by Ellis and associates (1926-1928). Slow gains may also be a factor, although Bull and Carroll (1928) report that the results of their work showed no indication that rate of gain had any effect on the type of carcase produced, while Martin (1928) reports that a decrease in the rate of gain resulted in a corresponding decrease in the percentage of firm carcases produced.

Summary of Results.

1. Only medium gains were obtained on all rations employed in this trial.

2. Feed consumption per unit of gain in weight was relatively high for the group of pigs on the maize-meal–meat-meal ration and fairly low for the groups on the rations containing barley.

3. There is some evidence to show that barley exercises a favourable influence on length of side, although insufficient to affect the grading of the carcases.

4. Slow gains, combined with relatively low killing weights, appear to be conducive to the production of a high percentage of No. 1 lean sizeable sides.

5. The hardening effect of barley in a ration consisting largely of maize is further confirmed by the results obtained in this trial.

6. Increasing the proportion of barley beyond 45 per cent. in the ration showed no additional effect on either texture of fat or length and grade of carcase produced.

7. The results obtained with the pigs on the maize-meal–meat-meal ration (Group I) indicate a probable connection between firmness of back-fat and age of the pig.

Acknowledgment.

Grateful acknowledgment is due to Mr. D. J. R. van Wyk, M.Sc., of the Division of Chemistry, for his technical assistance in connection with the refractive index determinations, and to the management of the Farmers' Co-operative Bacon Factory, Estcourt, for placing the facilities which their factory affords to the disposal of this Division for experimental purposes.

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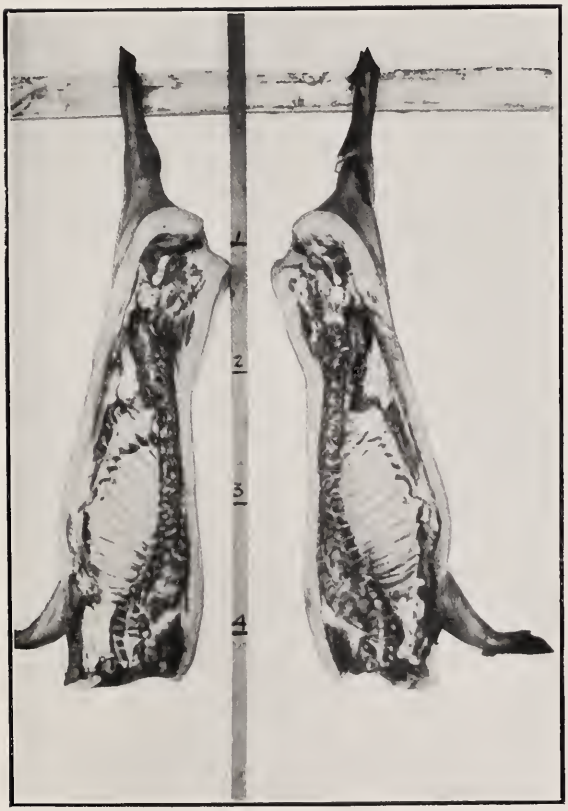

No. 1.—Lean Sizeable Bacon Side.

Planes of Feeding for Fattening Steers.

By D. J. SCHUTTE, B.S.A., M.Sc., and G. N. MURRAY, B.Sc.(Agric.), Division of Veterinary Services and Animal Industry.

THE experiments reported in this paper are a continuation of a series commenced in 1927 with the object of determining the most profitable methods of steer-feeding for the local market under conditions obtaining in the maize areas of the Union of South Africa.

REVIEW OF PREVIOUS TRIALS.

In the first trial of the present series, reported by Wande (1928), the following planes of feeding were employed:—

Lot I. Veld grazing only.

Lot II. Maize silage and teff-hay *ad lib.*

Lot III. Maize silage and teff-hay, plus a fairly heavy concentrate ration consisting of maize-meal, peanut-meal, and cotton-seed meal.

The length of the feeding period was 103 days and the average daily gains were: 0.50, 1.29, and 2.28 lb. for Lots I, II, and III respectively. It was concluded from the results obtained that the ration fed to Lot II was nearest the desirable plane of feeding. The most economical gains were made on this ration, and the difference in finish, and the margin in selling price between the steers in Lots II and III were insufficient to offset the cost of the additional feed consumed by Lot III.

In a subsequent trial of 105 days' duration, briefly reported by Romyn (1929), two lots of 10 grade Sussex steers were fed according to the following plan:—

Lot I. First period of 62 days: Teff-hay *ad lib.*; maize silage as much as they would clean up in an hour twice daily.

Remaining period of 43 days: Teff-hay and maize silage as above, plus shelled maize, 4 lb., and cotton-seed meal, 1 lb.

Lot II. First period of 43 days: Teff-hay and maize silage as above, plus cotton-seed meal, 1 lb.

Remaining period of 62 days: Teff-hay and maize silage as above, plus shelled maize up to 10 lb. and cotton-seed meal, 2 lb.

The results of this trial may be summarized as follows:—

TABLE I.

Summary of Results of 105 Days Feeding Period.

	Group I.	*Group II.*
Number of steers	10	10
Average initial weight (lb.)	1,013	1,017
Average final weight (lb.)	1,204	1,223
Average total gain (lb.)	190.4	205.5
Average daily gain during first period (lb.)	1.32	1.62
Average daily gain during second period (lb.)	2.75	2.50
Average daily gain for entire period (lb.)	1.85	1.99
Average daily ration (lb.)—		
Teff-hay	10.65	11.16
Maize silage	39.75	34.63
Shelled maize (for period fed)	4.51	6.80
Cotton-seed meal (for period fed)	1.65	1.88
Feed per 100 lb.-gain in live weight—		
Teff-hay (lb.)	576.2	559.1
Maize silage (lb.)	2,150.4	1,735.9
Shelled maize (lb.)	101.8	205.1
Cotton-seed meal (lb.)	37.3	94.8
Total roughage (lb.)	2,726.6	2,299.0
Total concentrates (lb.)	139.1	299.9

Table I shows that both groups made satisfactory gains. However, there was very little difference between the groups in respect of rate of gains. The steers in Group II gained significantly faster during the first period due to the additional maize and cotton-seed in the ration, but those in Group I made the better gains during the second period, consequently the final weights were very similar.

Group I consumed more roughage per unit of gain in live weight, but this was more than offset by the great saving in concentrates, and on the basis of relative prices for these classes of feeding stuffs Group I made the most economical gains.

The steers of both lots lacked finish at the end of the feeding period, but it was considered that they would all kill good medium to prime, with the exception of one animal in Group I.

There was practically no difference in the returns from the two lots after taking into consideration the profits obtained from the pigs which followed the respective groups.

It was concluded from the above trial that no advantage accrued from grain-feeding during the early stages of the feeding period, and that steers of the type, age, and condition used in this experiment should be fed a good roughage ration only during the first month of the feeding period, followed by the addition of a gradually increased allowance of grain and protein supplement.

PLAN OF EXPERIMENTS.

The experiments were carried out at the School of Agriculture and Experiment Station, Potchefstroom, Transvaal.

The feed-lot consisted of an open shed and yards. The steers were allowed to run free in the yards day and night and were only brought into the shed for feeding of silage and concentrates. Hay was fed from racks in the yards.

The grain ration was fed mixed with the silage and sterilized bonemeal at the rate of 4 oz. per animal daily was scattered over the silage. The animals had free access to water, and a salt-lick was provided from separate troughs in the pens.

The steers were weighed on three consecutive days at the commencement of the trials and thereafter at 30-day intervals.

At the conclusion of the respective trials, the animals were railed to Pretoria, a distance of approximately 120 miles, where they were slaughtered and the carcasses reported on.

Fig. 1.—Steers in open yard, showing hayrack. Note pigs running with the steers.

EXPERIMENT 1.

Steers Used.—Twenty grade steers (Shorthorn and Sussex), $3\frac{1}{2}$ to $4\frac{1}{2}$ years old, and selected from the same herd, were used in this trial.

Rations.—The steers were divided into two equal groups and fed for a period of 122 days, according to the following plan:—

First period of 60 days' duration—

Lot. I. Teff-hay *ad lib.* and maize silage as much as they would clean up in one hour twice daily.

Lot II. Teff-hay and maize silage as above, plus peanut-meal, 1 lb.

Second period of 62 days' duration—

Lot I. Same as during first period.

Lot II. Teff-hay and maize silage as above, plus shelled maize, 8 lb., and peanut-meal, 2 lb.

The steers were fed on these rations for a preliminary period of 10 days in order to get them accustomed to the feeds.

TABLE II.

LIVE WEIGHTS OF INDIVIDUAL STEERS FOR 120-DAY FEEDING PERIOD.

LOT I.

Steer No.	2	4	6	13	15	16	17	19	22	23	Average.
Initial weight (lb.)	1,007	895	1,017	997	938	858	1,078	1,020	922	883	961·5
Weight at end of 1st month	1,115	960	1,085	1,103	1,005	932	1,172	1,128	998	928	1,042·6
Total gains during 1st month	108	65	68	106	67	74	94	108	76	45	81·1
Weight at end of 2nd month	1,148	992	1,127	1,140	1,050	958	1 220	1,182	1,033	978	1,082·8
Total gains during 2nd month	33	32	42	37	45	26	48	54	35	50	40·2
Weight at end of 3rd month	1,183	1,000	1,132	1,172	1,017	1,063	1,222	1,198	1,045	1,005	1,103·7
Total gains during 3rd month	35	8	5	32	— 33	105	2	16	12	27	20·9
Final weight (122 days)	1,178	998	1,158	1,183	1,062	1,013	1,268	1,243	1,060	1,057	1,122·0
Total gains during final month	— 5	— 2	26	11	45	— 50	46	45	15	52	18·3
Total gains for 122-day period	171	103	141	186	124	155	190	223	138	174	160·5
Average daily gains (lb.)	1·40	0·84	1·16	1·52	1·02	1·27	1·56	1·83	1·13	1·43	1·32

LOT II.

Steer No.	1	3	7	8	9	12	14	18	20	21	Average.
Initial weight (lb.)	902	870	878	1,057	928	1,033	938	915	997	1,057	957·5
Weight at end of 1st month	967	1,017	943	1,112	938	1,098	1,018	1,033	1,127	1,145	1,039·8
Total gains during 1st month	65	147	65	55	10	65	80	118	130	80	82·3
Weight at end of 2nd month	1,075	1,062	968	1,162	1,002	1,156	1,023	1,105	1,188	1,212	1,095.3
Total gains during 2nd month	108	45	25	50	64	58	5	72	61	67	55·5
Weight at end of 3rd month	1,095	1,125	1,007	1,145	1,070	1,190	1,092	1,145	1,202	1,277	1,134·8
Total gains during 3rd month	20	63	39	— 17	68	34	69	40	14	65	39·5
Final weight (122 days)	1,182	1,213	1,095	1,232	1,175	1,338	1,197	1,250	1,325	1,357	1,236·4
Total gains during final month	87	88	88	87	105	148	105	105	123	80	101·6
Total gains for 122-day period	280	343	217	175	247	305	259	335	328	300	278·9
Average daily gains (lb.)	2·30	2·81	1·78	1·43	2·02	2·50	2·12	2·75	2·69	2·46	2·29

TABLE III.

Average Daily Feed Consumption per Steer (lb.).

Lot I.

Period.	*Hay.*	*Silage.*	*Shelled maize.*	*Peanut-meal.*
First period	14.6	17.5	—	—
Second period	11.1	37.3	—	—
Third period	5.2	44.3	—	—
Final period	5.6	53.1	—	—
Average for 122-day period	9.08	38.28	—	—

Lot II.

Period.	*Hay.*	*Silage.*	*Shelled maize.*	*Peanut-meal.*
First period	14.2	17.5	—	1.25
Second period	11.7	37.3	—	1.18
Third period	5.4	36.8	7.6	2.95
Fourth period	5.2	38.6	11.6	2.95
Average for 122-day period	9.01	32.63	9.68*	2.05

*Average for period during which fed.

TABLE IV.

Feed Consumption per 100-lb. Gain in Live Weight.

	Lot I.		*Lot II.*	
Feed.	*lb.*	*T.D.N.*	*lb.*	*T.D.N.*
Hay	690	331.20	396	190.08
Silage	2,907	514.54	1,428	252.76
Shelled maize	—	—	426*	358.70*
Peanut-meal	—	—	90*	75.15*
Total		845.74		876.69

*Calculated on gains for period during which fed.

Discussion of Results.

Referring to Table II, it will be observed that the steers of both lots made very good gains during the first 30-day period. This is accounted for by the fact that the steers were in rather thin condition, and that they put on weight rapidly at this stage. The consumption of roughage during this period was the same for both lots, as shown in Table III. The addition of a small amount of peanut-meal to the ration of Lot II during the first month did not appear to affect the gains of this lot in any way.

From the second month onward, the rate of gain for Lot I decreased progressively, while during the final 30-day period several steers actually lost in weight. The animals were induced to take silage to the limit of their appetites, and at the close of the trial the average daily consumption per steer was 53.1 lb. of silage and 5.6 lb. of hay, as compared with 17.5 lb. and 14.6 lb. respectively at the commencement of the trial.

The effect of the additional peanut-meal fed to Lot II is reflected in the larger gains made during the second month, as compared with Lot I. Beginning with the third month, shelled maize was introduced into the ration of Lot II, and the amount of peanut-meal offered was doubled. Although no immediate improvement resulted in the rate of gain for this lot, the full effect of this additional feeding is brought out by the figures for the last 30-day period, during which the average daily gain per steer was 3.18 lb. The feed consumption for this lot is shown in Table III. It will be observed that the steers consumed approximately the same amount of silage throughout the last 90 days of the trial, and that the hay consumption remained practically constant for the 60 days during which shelled maize was fed.

Table IV shows the feed requirements per 100 lb. of gain in live weight. According to Henry and Morrison (1923), steers of this class require 800 to 900 lb. of total digestible nutrients per 100-lb. gain. It is therefore apparent that the animals made good use of their feed.

The steers of Lot I utilized their feed to slightly better advantage on the average than those of Lot II.

Pigs.—Three weaner pigs averaging 66 lb. in weight were placed with each lot of steers. During the second months of the trial the number following Lot II steers was increased to 7. The pigs following Lot I consumed an additional amount of 822 lb. of grain for a total gain in weight of 209 lb. Those following Lot II consumed an additional 365 lb. of grain for a total gain in weight of 329 lb.—in other words, the additional grain required for 100 lb. gain in live weight for the pigs was 393 lb. and 111 lb. respectively. The pigs made very poor gains, especially during the first period; consequently, the undigested grain voided by the steers of Lot I did not appear to have effected an appreciable saving in the amount of feed required per 100-lb. gain. On the other hand, the pigs following Lot II showed a considerable saving in grain, approximately 200 lb. per 100-lb. gain in live weight.

TABLE V.

Slaughtering Data and Carcass Reports.

	Lot I.	*Lot II.*
Average shrunk weight (lb.)	1,023.0	1,154.5
Average shrinkage (per cent.)	8.8	6.6
Average dressed weight (lb.)	554.0	656.0
Average dressing percentage	54.2	56.8
Average finish, per cent., prime	10.0	60.0
Average per cent.—		
Good medium (choice)	10.0	40.0
Medium	40.0	—
Poor	20.0	—

Lot I showed a greater shrinkage in transit than Lot II due probably to the unfinished condition of the former. The steers of Lot II likewise had a higher dressing percentage. The great difference in finish between the lots is shown by the grading; all the steers of Lot II graded prime or choice, whereas only two in Lot I graded prime or choice, while the rest carried only medium condition. These differences were also observed in the quality of the beef. The steers of Lot II showed a uniform covering of good-coloured fat and were superior in marbling to those of Lot I.

Financial Returns.

Since the financial outcome of an experiment of this nature depends upon so many contributing factors which are not under experimental control no attempt will be made to present a logical statement of financial results. However, for the purpose of comparison, a statement of the cost of feed and revenue produced is given for the respective lots. No account is taken of such factors as labour, manure, etc.

TABLE VI.

Feed Cost and Revenue Produced.

Feed Cost.	*Lot I.*	*Lot II.*
Silage, at 10s. per ton	£11 13 6.6	£9 19 0.6
Teff-hay at 70s. per ton	19 7 11.7	19 6 10.3
Maize, at 4s. 6d. per 100 lb.	—	13 10 0
Peanut-meal, at 11s. 3d. per 100 lb.	—	14 1 3
Bonemeal, at 9s. 9d. per 100 lb.	1 9 0.7	1 9 0.7
Salt, at 2s. 4½d. per 100 lb.	0 1 10.4	0 1 10.4
Total	£32 12 5.4	£58 8 1
Average cost of feed per steer	£3 5 3	£5 16 9.5

Revenue.

	Lot I.	*Lot II.*
592 lb. prime beef, at 40s. per 100 lb.	£11 16 0	
592 lb. good medium beef, at 37s. 6d. per 100 lb.	11 2 0	
3,221 lb. medium beef, at 32s. 6d. per 100 lb.	52 6 4	
970 lb. poor beef, at 30s. per 100 lb.	14 11 0	
3,995 lb. prime beef, at 40s. per 100 lb.	—	£79 18 5
2,367 lb. good medium beef, at 37s. 6d. per 100 lb.	—	44 7 1
Total revenue	89 15 4	124 5 6
Average revenue per steer	8 19 6	12 8 6
Average pig profit per steer	—	0 5 1
Total revenue per steer	8 19 6	12 13 7
Average return per steer above feed cost	5 14 3	6 16 9

A comparison of the net returns per steer above feed cost for Lots I and II respectively shows that the steers of Lot II more than repaid for the extra feed which they received.

The results of this experiment indicate that steers of the age and type used in this experiment could not be finished on roughage supplemented with a small amount protein supplement during the latter half only of a feeding period of 122 days' duration. The addition of a fairly liberal allowance of shelled maize and a small amount of protein supplement to a basal ration consisting of teff-hay and maize silage, resulted in a great improvement in condition of the steers and the selling price was enhanced accordingly.

EXPERIMENT II.

Steers Used.—Sixteen Hereford × Afrikander and Sussex × Afrikander cross-bred steers, ranging in age from $3\frac{1}{2}$ to $4\frac{1}{2}$ years, were used in this experiment.

Rations.—In view of the results obtained in Experiment I above, a higher plane for feeding was employed in the present trial. The following average rations were fed to two equal groups of eight steers each for a feeding period of 102 days' duration:—

First period of 30 days' duration—

Lot I. Teff-hay, approximately 10 lb. Maize silage as much as the steers would clean up in an hour twice daily.

Lot II. Teff-hay, as above. Maize silage, as above, plus shelled maize, 1·5 lb.

Second period of 30 days' duration—

Lot I. Teff-hay, as above. Maize silage, as above, plus shelled maize, 1–5 lb. Peanut-meal, 1.5 lb.

Lot II. Same as during first period.

Third period of 42 days' duration—

Lot I. Teff-hay, as above. Maize silage, as above. Shelled maize, 10 lb. Peanut-meal, 1.5 lb.

Lot II. Teff-hay, as above. Maize silage, as above. Shelled maize, 10 lb. Peanut-meal, 1.5 lb.

TABLE VII.

LIVE WEIGHTS, ETC., FOR 102-DAY FEEDING PERIOD.

LOT I.

Steer No.	51	57	92	10H	9	15	18	19	Average.
Initial weight (lb.)	1,210	1,142	1,282	1,030	987	1,018	1,020	942	1,078·8
Weight at end of 1st month	1,305	1,213	1,373	1,127	1,098	1,183	1,105	1,068	1,184·1
Total gains during 1st month	95	71	91	97	111	165	85	126	105·1
Weight at end of 2nd month	1,320	1,260	1,420	1,148	1,155	1,172	1,168	1,097	1,217·5
Total gains during 2nd month	15	47	47	21	57	— 11	63	29	33·5
Final weight (102 days)	1,453	1,265	1,488	1,238	1,230	1,283	1,265	1,145	1,295·9
Total gains during final period	133	5	68	90	85	111	97	48	79·6
Total gains for 102-day period	243	123	206	208	243	265	245	203	217·1
Average daily gains (lb.)	2·38	1·19	2·02	2·04	2·38	2·60	2·40	1·99	2·13

LOT II.

Steer No.	33	37	49	7	14	10	17	20	Average.
Initial weight (lb.)	1,317	1,228	1,115	988	1,097	1,020	970	960	1,086·9
Weight at end of 1st month	1,435	1,307	1,202	1,098	1,203	1,117	1,067	1,028	1,182·1
Total gains during 1st month	118	79	87	110	104	97	97	68	95·0
Weight at end of 2nd month	1,478	1,372	1,253	1,102	1,225	1,193	1,097	—	1,245·7
Total gains during 2nd month	43	65	51	4	22	76	30	—	41·4
Final weight (102 days)	1,588	1,430	1,310	1,230	1,310	1,288	1,173	—	1,332·7
Total gains during final period	110	58	57	128	85	95	76	—	87·0
Total gains for 102-day period	271	202	195	242	213	268	203	—	227·7
Average daily gains (lb.)	2·66	1·98	1·91	2·37	2·09	2·64	1·99	—	2·23

TABLE VIII.

AVERAGE DAILY FEED CONSUMPTION (LB.).

LOT I.

Period.	*Hay.*	*Silage.*	*Maize.*	*Peanut-meal.*
First period	10.6	46.8	—	—
Second period	8.1	45.5	4.4	1.7
Final period	9.7	36.6	9.6	1.4
Average for 102-day period...	9.4	41.8	5.9*	1.2*

*Average for period during which fed.

LOT II.

Period.	*Hay.*	*Silage.*	*Maize.*	*Peanut-meal.*
First period	10.5	39.2	4.8	—
Second period	7.3	39.2	9.2	1.7
Final period	9.6	34.0	9.9	1.4
Average for 102-day period...	9.2	37.5	8.2	1.1

TABLE IX.

FEED CONSUMPTION PER 100-LB. GAIN IN LIVE WEIGHT.

	Lot I.		*Lot II.*	
Feeding Stuff.	*lb.*	*T.D.N.*	*lb.*	*T.D.N.*
Hay	443.9	215.3	414.5	201.0
Silage	1 963.6	347.7	1,676.5	296.8
Shelled maize ...	484.5*	387.5*	368.8	295.0
Peanut-meal	100.7*	83.7*	49.3	41.2
Total		1,034.2		834.0

* Calculated on gains for period during which fed.

DISCUSSION OF RESULTS.

It is interesting to note from the figures in Table VII that the best gains were made by both lots during the first month of the feeding period. The difference in daily rate of gain between the two lots for this period was 0.34 lb. in favour of Lot I, which received no grain. This difference is too small, however, to be of much significance, and it may be taken to indicate that the addition of grain during the initial stages of the feeding period is not justified in the case of relatively thin store animals. From the second period until the end of the trial the steers of Lot II maintained a slight advantage in respect of rate of gain over those of Lot I.

The marked drop in gains during the second month is explained by the fact that all the animals were inoculated against anthrax at the beginning of the second 30-day period. The animals reacted very severely to the inoculation; one steer in Lot II developed a stiff neck and lost in weight. It failed to recover and had to be removed from the experiment. As the animals recovered from the effects of the inoculation, the daily rate of gain improved accordingly. During the last ten days of the trial, the average daily gains

were 3.77 lb. and 2.97 lb. respectively. The average daily feed consumption is set forth in Table VIII. It will be observed that the consumption of teff-hay was similar for both lots throughout the trial. The steer of Lot II consumed a samewhat smaller amount of silage than those of Lot I, due no doubt to the heavier allowance of grain in the ration of the former.

Table IX indicates that the steers of Lot II apparently utilized their feed more economically than those of Lot I. This difference is due largely to the fact that the poorest gains were made for the period during which grain was fed to the latter group since the amount of grain consumed is only credited with the gains actually made during that period. When credited with the gains for the entire feeding period the amount of shelled maize and peanut-meal required per 100-lb. gain in weight becomes 249.4 lb. and 51.8 lb. respectively. On this basis, the total digestible nutrients per 100-lb. gain becomes 805.8 lb., which is even lower than that for Lot II.

Pigs.—Six Tamworth gilts, averaging 80 lb. in live weight, were placed with the Lot II steers from the commencement of the experiment. No pigs were run with the Lot I steers until the beginning of the second month of the trial, when shelled maize was added to the ration of this lot, since it was found in Experiment I described above that the amount of wasted grain in silage alone saved very little in the extra feed to be fed to the pigs. Separated milk, at the rate of .75 gallons per pig, was fed to the pigs following both lots, except during the last 12 days of the trial, when meatmeal was substituted for the milk. This amount was considered just sufficient to balance the grain picked up by the pigs.

The pigs following Lot II steers made excellent gains and reached baconer weights after 70 days in the feed lot, when they were replaced by a further eight Tamworth pigs of 50 lb. average live weight.

Six pigs were run with the steers of Lot I from the beginning of the second month.

The pigs of Lot I only consumed an additional 100 gallons of separated milk and 34 lb. of meatmeal for a total gain of 351 lb., showing a saving in grain equivalent to approximately 880 lb. of maize. The pigs of Lot II consumed an additional 190 gallons of separated milk and 34 lb. of meatmeal for a total gain of 785 lb. of pork, which represents a saving of 1,960 lb. of maize.*

These results are of great significance as indicating the large amount of undigested grain voided by the steers [25 per cent. and 28 per cent. of the total grain (excluding that obtained from the silage) fed to the steers of Lots I and II respectively], and emphasizing the importance of the pig profits in any system of economical steer feeding.

* Based on the feed requirements per 100 lb. gain in live weight of baconers as determined in the pig-feeding experiments carried out by the Division of Veterinary Services and Animal Industry.

TABLE X.

Slaughtering Data and Carcass Reports.

	Lot I.	*Lot II.*
Average shrunk weight (lb.)	1,119	1,154
Average shrinkage (per cent.)	10.52	11.18
Average dressed weight (lb.)	670.0	673.0
Average dressing percentage	59.92	58.11
Finish, per cent., prime	37.5	43.0
Per cent.—		
Good medium (choice)	50.0	57.0
Medium	12.5	—
Poor	—	—

Lot II shows a slightly higher shrinkage and a lower dressing percentage than Lot I. The differences are, however, very small. The two lots were very similar in all respects at the end of the trial. Only very slight differences in finish between the steers of the different lots could be detected.

The prime carcasses of both groups showed good quality, and there was practically no difference in this respect which could be attributed to the effects of different rations. The marbling and the proportion of fat to lean were very desirable. The medium carcasses showed a lack of quality in the poor covering and bluish colour. The marbling was only medium and, in the case of steer No. 15, quite deficient. The medium grade carcasses of Lot II showed up better than those of the other group, and it was apparent that the additional maize in the ration of these steers effected an improvement in the quality of the carcasses, but not to an extent sufficient to raise the grade.

TABLE XI.

Feed Cost and Revenue Produced.

Feed Cost.	*Lot I.*	*Lot II.*
Silage, at 10s. per ton	£8 10 6	£7 12 9
Teff-hay, at 70s. per ton	13 9 9	13 4 0
Maize, at 4s. 6d. per 100 lb.	9 14 11	15 2 4
Peanut-meal, at 11s. 3d. per 100 lb.	5 1 3	5 1 3
Bonemeal, at 9s. 9d. (per 100 lb.)	0 19 6	0 19 6
Salt, at 2s. 4½d. per 100 lb.	0 1 6	0 1 6
Total	£37 15 5	£42 1 4
Average cost of feed per steer	£4 14 5	£5 5 2

Revenue.

Lot I.

2,240 lb. prime beef, at 40s. per 100 lb.	£44 16 0
2,480 lb. good medium beef, at 37s. 6d. per 100 lb.	43 10 0
640 lb. medium beef, at 32s. 6d. per 100 lb.	10 8 0

Lot II.

2,340 lb. prime beef, at 40s. per 100 lb.	—	£46 16 0
2,374 lb. good medium beef, at 37s. 6d. per 100 lb. ...	—	44 10 3
Total	£98 14 0	91 6 3
Average revenue per steer	12 6 9	13 0 11
Average pig profit per steer	0 5 0	0 11 0
Total revenue per steer	12 11 0	13 11 11
Average return per steer above feed cost	7 17 4	8 6 9

The difference in average return above feed cost between the lots is very small, and the slight advantage shown by Lot II is largely due to the increased profits derived from the pig. It is apparent, therefore, that very little was gained by the feeding of grain to the steers of Lot II during the first month of the trial. It is significant, however, that a high percentage of steers in both lots were in an unfinished condition at the end of the experiment, and it would appear that even under conditions of fairly heavy grain feeding, a longer feeding period is required than that employed in this trial. It is interesting to note that all the older steers graded prime and the younger ones only graded medium. Incidentally, the former class comprised all Hereford × Afrikander cross and the latter Sussex × Afrikander.

These observations are confirmed to some extent by the results of some overseas investigations. For example, in a series of feeding trials extending over a period of six years, in which different planes of feeding were compared, Tomhave and co-workers (1923) found that steers fed maize, silage, maize-stover, shelled maize, and cotton-seed meal gained only .28 lb. more per head daily than those receiving no maize, and .15 lb. more than those fed shelled maize during the latter half of the feeding period. The market finish for the steers fed the limited corn ration was almost as good as for the steers fed maize for the entire period, and the former actually returned five dollars and 48 cents more per head. These results are in close agreement with those obtained in the trials reported in this paper, and the results of five feeding trials carried out to date indicate fairly definitely that store cattle in relatively poor condition will make very efficient use of a ration consisting solely of roughage such as hay and silage during the first month in the feed lot. It is also apparent that no particular advantage accrued from heavy grain feeding from the second month onwards. It is only after the animals have reached a fair degree of finish that heavy grain feeding should be resorted to in order to finish them off.

These conclusions apply particularly to older steers, i.e. 3½ to 4 years of age, and it is doubtful whether 2-year-old animals could be fattened economically on such a limited grain ration. It would appear that young steers require a much longer feeding period, since it was observed in each of the above trials that the 2 to 3 year old steers were relatively unfinished at the end of the trial in spite of gains in weight comparable to that of the older animals. Similar results were obtained by Bohstedt (1922) and several others. Increase in weight of young steers is apt to be due to growth rather than finish, consequently such animals require a decidedly fattening ration unless the feeding is extended over long periods.

SUMMARY.

1. Two steer-feeding trials, including several planes of feeding, are reported in this paper.

2. No increase in gains resulted from the addition of grain and concentrates during the first month of the feeding period to a good roughage ration consisting of maize silage and teff-hay.

3. A medium grain supplement added during the second month of the feeding period, proved more economical than silage and hay, either alone or supplemented with a small amount of peanut-meal.

4. A medium grain supplement during the second month proved more economical than a heavy grain supplement.

5. A fairly heavy grain ration is necessary towards the end of the feeding period in order to ensure a desirable degree of finish in the steers.

6. The results indicate that 2 to 3 year old steers require a longer feeding period than is required by 3 to 4 year old animals.

7. A feeding period of 100 to 120 days' duration was insufficient for finishing off the type of steers used in these trials.

8. Less pork was produced on the limited grain rations, while the running of pigs with steers receiving no grain except that which is provided by the silage, proved unprofitable.

ACKNOWLEDGMENT.

Acknowledgment is due to Mr. C. A. Murray, B.Sc.(Agric.), for his assistance in connection with Experiment I and to the Transvaal Koelkamers, Ltd., Pretoria, for kindly providing the necessary facilities for slaughter and grading of the carcasses.

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Fig. 2.—Type of Sussex grade steer used in Experiment II. Steer No. 10 at commencement of trial.

Fig. 3.—Type of Sussex grade steer used in Experiment II. Steer No. 10 at conclusion of trial.

Fig. 4.—Type of Hereford×Afrikander crossbred steer (No. 92) used in Experiment II, at commencement of the feeding trial.

Fig. 5.—Type of Hereford×Afrikander crossbred steer (No. 92) used in Experiment II, at the conclusion of the following trial.

Fig. 6.—Carcasses of four steers in Lot I, Experiment I.

Fig. 7.—Carcasses of four steers in Lot II, Experiment I.

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The Causes of Variation in the Fat Content in Successive Portions of Milk.

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(Extracted from a Masters Thesis submitted to the Graduate Faculty at Ames, U.S.A.)

The increase in the fat-content in successive fractions of the milk at each milking is universally recognized. That this fact has been known for more than two hundred years is indicated by Allen (2). In referring to the West Highland practices of 1723, Allen says: " In summer all the cattle are pastured; the calves are sent to their dams twice in the day, and the strippings or last part of the milk, is taken away by the dairy maid, for it is commonly supposed that, if the calf is allowed to draw all the milk he can, it will keep the dam in low condition, and prevent her being in calf in proper time. . . . At each milking, as long as milk is given, that which is drawn off first, is more watery than that which is obtained last."

The great advancement of science during the last century has afforded some plausible explanations for the increased fat-percentages in successive fractions of milk drawn from the mammary gland. Much remains, however, to be learned about this complex phenomenon.

Physiologists are well aware of the wonderfully efficient and intricate body mechanisms whereby nature strives to maintain the normal healthy body balance. It is, therefore, obvious that she would not allow the strippings to be so much richer than the first drawn milk without a very good objective. Future investigators will endeavour to explain what beneficial effect such a condition in the milk flow has upon the young.

The work reported herewith is an attempt to explain the condition in which the milk exists in the udder after it has been secreted and before it is withdrawn, with the hope of throwing some light upon the causes of the ultimate variation in fat-content in succesive samples drawn at a milking.

Review of Previous Investigations.

The subject, milk secretion, has been studied from various angles by different investigators. Physiological studies have not received consideration here, because the quantity of such work would be beyond the scope of this thesis. The work of other investigators may be grouped under four general headings.

A. The Variations in Butter-fat Test in Different Portions of a Milking.

B. The Variations in Solids-not-fat in Different Portions of a Milking.

C. The Variations in Milk and Fat Yields of the Different Quarters of a Cow's Udder.

D. The Theories for the Variation in Butter-fat Test in Different Portions of a Milking:—

(i) A gravity separation of the fat globules already secreted in the udder.

(ii) A slower liberation of the larger fat globules.

(iii) A reabsorption of the solids-not-fat-producing mechanism.

(iv) A fat-producing mechanism and a lactose-producing mechanism.

(v) An increase in fat-percentage due to the stimulative effect of manipulating the udder.

(vi) A continuous liberation of milk into the udder.

The Objects of this Work.

The objects of the experiment reported here are:—

(1) To measure the variation in the percentage of the fat in different fractions of the milk from a milking.

(2) To note the effect upon the fat-percentage of massaging the udder just previous to milking.

(3) To note the effect upon the fat-percentage of agitation of the udder by vigorous exercise just previous to milking.

(4) To study the effect of the animal's position during or previous to milking upon the fat-percentage of the milk.

(5) To determine the variation in the solids-not-fat in successive portions of the milk from a milking.

(6) To ascertain any possible correlation between the rates of secretion and the percentage of fat in the milk.

The Method of Procedure.

In order that the results might be as representative as possible, cows of the Ayrshire, Jersey, Guernsey and Friesland breeds were used in this work. Normal healthy individuals were selected. It was desired that animals obtained give a fair amount of milk, in order that a large number of samples could be obtained at a milking from an individual.

In addition to the six cows, a healthy two year old grade Saanen doe, giving two pounds of milk daily, was used in this experiment.

All cows received the same treatments throughout the different experimental periods. They were kept with the rest of the herd, milked and fed with the herd, and every effort was made toward regularity in order and time of milking. The samples were collected at the evening milking and tested by means of the Babcock test the next morning. The Quevenne lactometer was used to determine the specific gravity of each sample.

The solids-not-fat was then determined by the following formula: —

$$\frac{L \div F}{4} = S.N.F.$$

L = Lactometer reading.
F = Butter-fat percentage.

Method of Computing Results.

No animal will yield a uniformly exact amount of milk at each milking. This fact resulted in a variable number of samples for the different milkings from an individual. Due to the collection of foam in the sample jars, weights of individual samples were often variable too. In certain periods it was considered advisable to discard every other sample. To put all these variables on a comparable basis, an effort was made to devise some way of presenting a truer representation than could be obtained from a simple average of an uneven number of samples. The plan adopted is best studied from Table I.

TABLE I.

Cow No. 749 after standing still.

Date.	Sample No.	Weight of Sample in Decigrams.	Percent of Total Weight of Milking.	Total Accumulative Percentage.	Mid Point of Accumulative Percent of Milking.	Butter Fat Test.
1/8/29	1	46	8·8	8·8	4·4	3·0
	2	45	8·6	17·4	—	—
	3	40	7·7	25·1	21·2	4·1
	4	45	8·6	33·7	—	—
	5	44	8·4	72·1	37·9	4·6
	6	45	8·6	50·7	—	—
	7	44	8·4	67·7	54·9	4·7
	8	55	8·6	76·3	—	—
	9	45	8·6	84·9	72·0	5·5
	10	45	8·6	84·9	—	—
	11	44	8·4	93·3	89·1	5·6
	12	34	6·5	99·8	96·5	6·7
		522	99·8			

It will be remembered that in period II every alternate sample was discarded, except when it happened to be the last sample of that milking. This procedure is clearly illustrated in the last two columns of Table I.

The weight of each sample multiplied by 100 is divided by the total weight of all samples in the milking (522 decigrams in this case), to determine the percentage of the whole milking, which that sample represents. By accumulation the total percentage of the milk obtained up to and including a certain sample, is determined. It is desirable to plot the fat tests at the mid points of accumulative samples. For example, sample No. 3 in Table I represents a fraction of the milking taken from 17.4 to 25.1 per cent. of the whole, the mid point of this fraction is 21.2 per cent. The mid points so plotted from the samples in the milking were connected by straight lines. Line A on Fig. 1 was plotted in this manner from data in Table I. Similar data from two other milkings from this cow were likewise plotted on Fig. 1.

Selecting arbitrarily points on the abscissa at 10 per cent. and at multiples thereof which points represent fractions of the milking, averages were obtained by reading from the chart the points where the three curves so constructed crossed the lines of each 10 per cent. of the milking. For example, it will be observed that the curves cross the 10 per cent. line at 3.40, 2.68 and 2.57. The average of these three points has been plotted with other averages similarly obtained.

The point on the 10 per cent. line represents only the fat-test at the moment when 10 per cent. of the milking had been obtained. Similar meanings are attached to the other points.

In this manner charts were constructed and averages obtained for each treatment of each animal. These individual charts were necessary in the compilation of the data which are indicated in Fig. 2. It was not, however, necessary to present these individual charts here.

Results.

(1) *Fat in the Different Portions of a Milking.*

(*a*) *After the Cows had been kept standing still in Stanchions.*

Samples were taken at 44 different milkings when the animals had been standing still in their stanchions. In 91 per cent. of these milkings a distinct rise in fat-test for each successive sample after the first drawn was observed. In only 9 per cent. of the observations did the curve reverse its usual formation. Due to individual variations in the fat tests, variations in quantity of milk given, temperatures and other factors usually influencing production around all barns the individual curves for each milking show slight variations. The average fat tests of all successively drawn samples in 44 milkings after the cows had been standing still, are presented in Fig. 2.

The average butter-fat test is 1.95 per cent. for the first drawn milk and 7.24 per cent. for the last drawn milk.

It will be noticed that there is an appreciable rise in the fat test during the first 40 per cent. of the milk drawn. The rise is not so great during the next 40 per cent. of the milk drawn, but is most rapid for the last 20 per cent. of the milking. It is no uncommon occurrence for the last sample to test as high as 2 per cent. above that of the immediately previous sample.

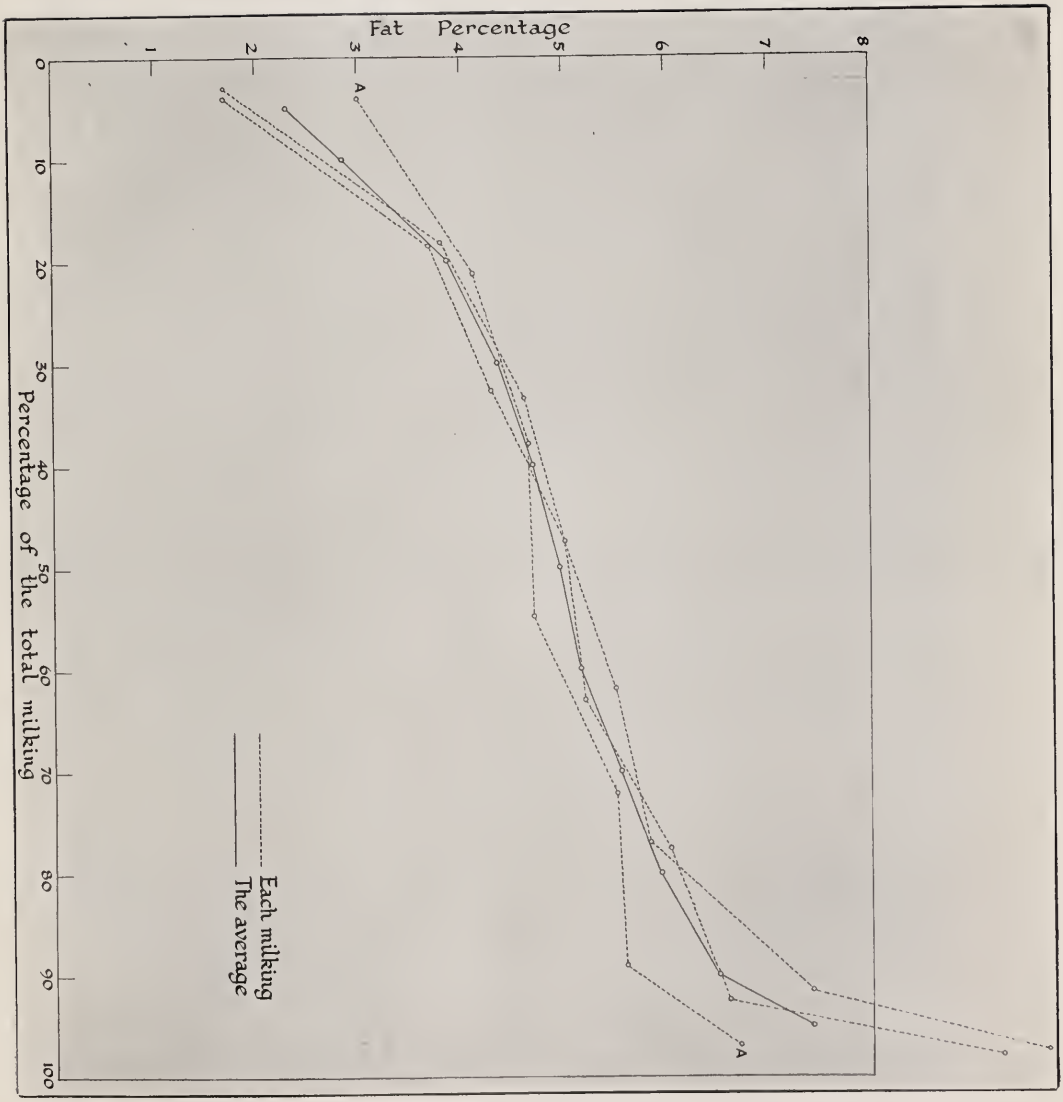

Fig. 1.—Cow No. 749 After Standing Still.

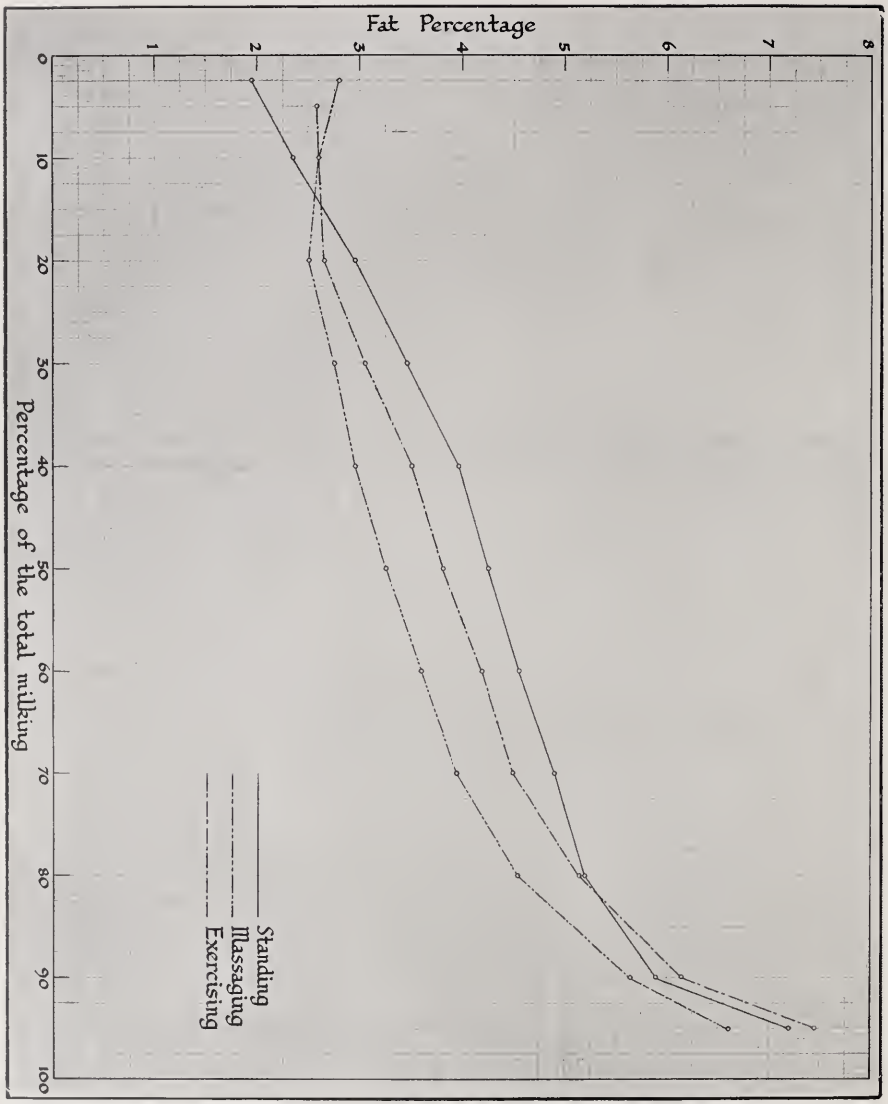

Fig. 2.—Fat Tests of Successive Percentages of the Total Milking.

(*b*) *After Massaging the Udders just previous to Milking.*

A total of 51 observations were made on the fat tests of successive samples after the udder of the cow had been massaged. The curve of these individual milkings reveal some startingly wide variations. Some individual cows such as 849, hardly respond to the treatment at all, as may be seen in Fig. 3 which represents an average of the three milkings obtained in period 2 after massaging the udder.

The curve of Fig. 3 conforms quite closely to that illustrating the fat tests of samples taken after the cows had been standing still in their stanchions.

When an average is obtained for all successive sample fat tests, however, the curve shown in Fig. 2 is derived. There is a marked difference between the fat tests of successive samples of milk drawn after massaging the animal's udder and those tests obtained after the animals stood still before the milk was drawn.

After massaging the udder, sample No. 1 tests higher than sample No. I after the cow stands still, and is followed by a lower test for sample No. 2. The differences in the fat tests are not so marked between the individual samples when the udder has been massaged. The curve has a general scooping swerve as seen in Fig. 2. According to this curve the first four samples are influenced most by this treatment. The tendency being to equal their butter-fat tests.

(*c*) *After Exercising the Cows just previous to Milking.*

The cows were thoroughly exercised on 33 occasions and again individual variations were noticeable. Fig. 2 shows the average of all tests when the cows had been exercised.

Two very important points are illustrated in this curve:—

(i) The first two samples are evened up as regards their fat test.

(ii) The last sample drawn tested .88 per cent. higher than when the cows stood still previous to milking.

(2) *Studies on a Goat.*

The goat was an extraordinarily docile and patient animal, and could be easily handled at all times. At first she was given treatment similar to that given the cows. On 7 different occasions her udder was massaged before milked, while on 7 other occasions she stood still before milked.

The curves representing these treatments are in Fig. 4. In case of massaging all samples have approximately the same test, broken only in the high test of the last sample. Standing still, on the other hand, shows an increased fat test for each successive sample.

The goat was placed on her back on 8 different occasions, and all the samples found to test approximately the same, although these tests were not as uniform as when the udder of the doe had been massaged. After she had been placed on her back and immediately milked, a slight rise for each successive milk sample was noticeable as is shown in Fig. 4. Abnormally low tests were observed after

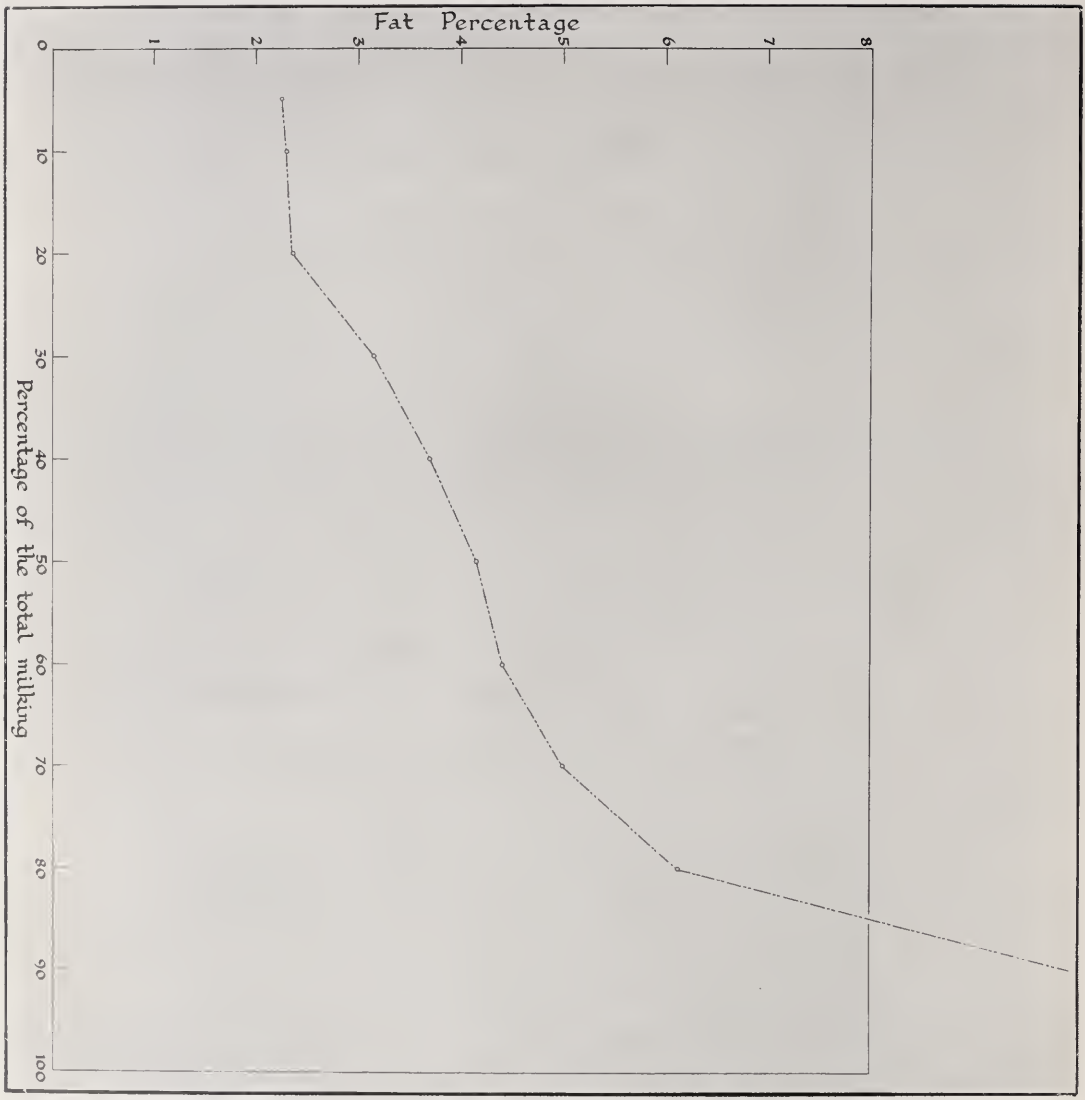

Fig. 3.—Cow No. 849 After her Udder was Massaged.

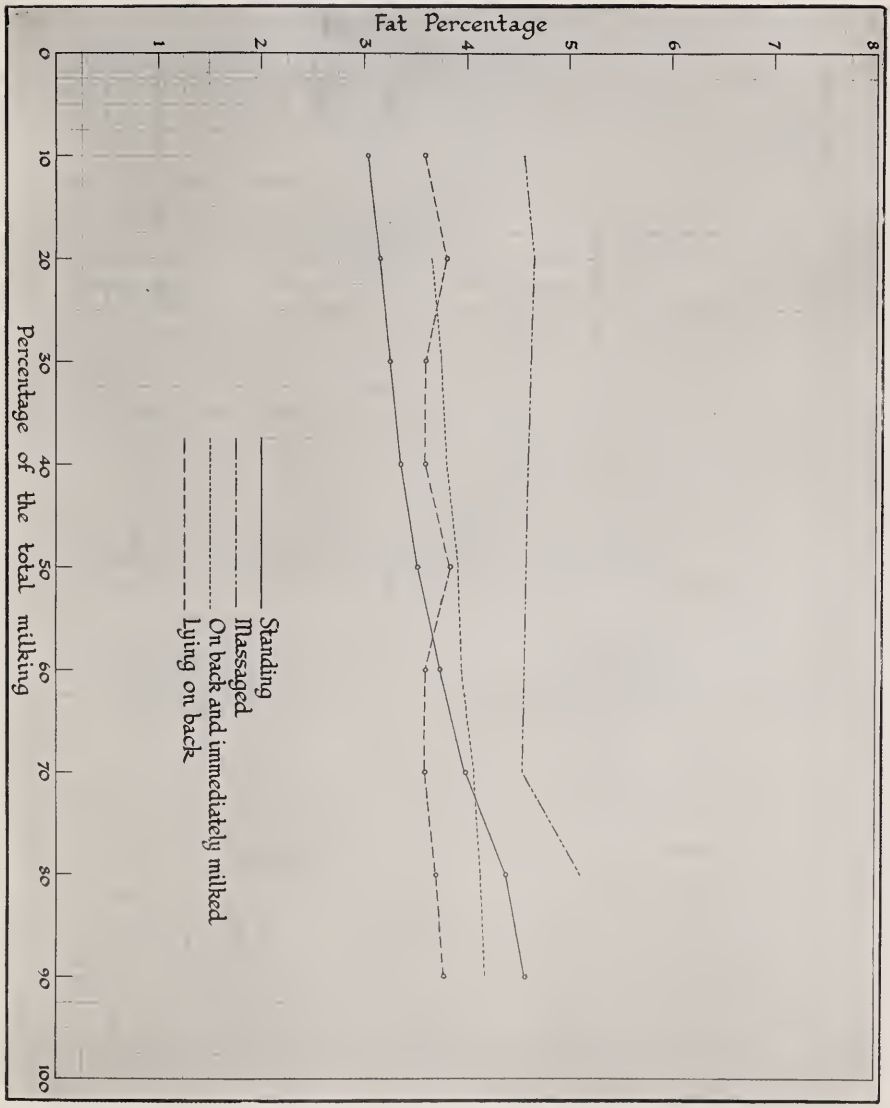

Fig. 4.—Fat Tests of Successive Percentages of the Total Milking from a Goat.

milk had been allowed to accumulate in the udder of the doe. The kid was allowed to nurse and so empty the udder, and then the doe was made to lie on her back for five hours before milking her. The low tests so obtained led to a different procedure. The goat was placed on her back for three hours, then one teat was milked, supposedly dry. She was then allowed to get up and the kid allowed to nurse the other teat. After waiting half an hour the same teat from which the samples were obtained was again milked. The results are given in Table II.

TABLE II.

Per cent. Fat from Succesive Samples of the Goat.

Sample.	On her Back for 2 Hours' Test.	Sample.	After Waiting ½ an Hour Test.
1	3·6	1	5·4
2	3·8	2	5·4
3	3·6	3	5·6
4	3·6	4	5·8
5	3·4		
6	3·3		
7	3·6		

As was expected, the tests were nearly 2 per cent. higher in the second milking after she was allowed to stand for half an hour.

(3) *The Solids-not-fat in Different Portions of a Milking.*

Table III has been compiled so as to present the average per cent. of all solids-not-fat in successive samples of a milking. The averages for all samples for each treatment is also given, as well as an average for all samples for all treatments.

TABLE III.

Cow.	Manipulation.	Average Solids-not-fat in Successive Samples.	Average for each Treatment.	Average for all Treatments.
736	Standing	9·63–9·70–9·68–9·71–9·55	9·66	9·81
	Massaging	9·66–9·71–10·68–9·86	10·01	
	Exercising	9·31–9·71–9·89–10·15–9·55	9·72	
863	Standing	9·20–9·64–9·53–9·47–9·41		
		9·52–9·70–9·15–9·10–9·27	9·31	9·53
	Massaging	9·40–9·73–9·50–9·63–9·45		
		9·65–9·49–9·36–9·36	9·53	
833	Standing	7·67–7·47–7·94–8·34–8·69		
		8·55–8·54–8·67–8·98	8·27	8·49
	Massaging	8·50–8·50–8·54–8·67–8·45		
		8·55–8·57–8·44–8·61–8·45	8·54	
	Exercising	8·44–8·81–8·90–8·79–9·00		
		8·79–8·45–8·49	8·69	

The foregoing tables show that there is not much difference in the percentage of solids-not-fat in different portions of a milking.

Where slight variations occur, these were undoubtedly due to experimental error, or the extremely wide ranges of temperature at the time of taking the lactometer readings.

The Holstein had an average of 1 per cent. less solids-not-fat than the Jersey and Guernsey.

(4) *The Correlation between the Rate of Milk Flow and the Fat-percentages.*

TABLE IV.

Correlations.

Cow No.	736	835	749	570
Standing.....	·1089 ± ·2007	·5375 ± ·0907	·0134 ± ·1509	·5375 ± ·0907
Massaging.....	·1475 ± ·2303	·5151 ± ·1033	·1342 ± ·1578	·5151 ± ·1033
Exercising.....	·7816 ± ·1071	·2363 ± ·1225	·4915 ± ·1044	·6644 ± ·0843

Observations show that the milk flow was most rapid during the middle of a milking. The first and last few samples always took longer to be drawn, than did those of the middle portion.

Interpretation of Results.

It seems reasonable to assume that the following processes all combined, influence the variation of the fat-percentage in different parts of the udder:—

(1) There is a creaming process in the larger milk cisterns.

(2) The larger fat globules are retained in the narrower ducts and lobules of the udder. In support of the above supposition, the following facts are cited:—

(i) The curve, illustrating tests after the animal had been standing still, shows a steady rise for the first four samples (Fig. 2). Then a point is reached where the curve bends slightly towards the horizontal plane, before making a sharp upward turn for the last two samples. This shows that a creaming process must have been going on in the large cisterns above the teats, while a considerable amount of butter-fat was present in the upper part of the udder as well.

(ii) When the udder is massaged, there is a tendency to equalize the tests of the first samples. The success depends upon the udder structure and the behaviour of the individual (Fig. 3). In massaging, however, there is always a tendency of the last sample to test higher than other samples tested, because the larger fat globules are dislodged from the narrow ducts.

(iii) When the animal is exercised, the first two samples have nearly the same percentage fat tests, due no doubt to the disturbance of the milk in the milk cisterns. On the other hand, the last sample tests slightly higher than for any other treatment. This is such a small percentage that it would be of no practical importance, yet it is an indication that the larger fat globules have been brought down in the process of exercising (Fig. 2).

(iv) When the goat is turned on her back and milked immediately. The fat tests increase for every successive sample. After she has been on her back a while, however, all the samples test practically the same. This again demonstrates the creaming process (Fig. 4).

(v) When the goat is kept on her back for three hours, milked and then allowed to stand in her pen for half an hour before again milking the same teat, the samples obtained from the second milking test 2 per cent. higher than the tests for the first drawn samples (Table II). This is conclusive evidence that the fat is retained in the upper portion of the mammary gland.

(vi) A portion of the milk may be secreted during the milking process without interfering with the distribution of the fat already secreted in the udder. Increased fat production due to udder stimulation could not be detected in any of the observations. Whenever the fat percentages were increased the causes could be ascribed to mechanical udder agitation, whereby the larger fat globules had become dislodged from the narrower ducts. The fact that the fat percentage of the first drawn sample might be increased by agitation or massaging, also disproves the theory that the milk which has been in the reservoir for some time has had part of its contained fat reabsorbed by the lymphatics.

With regard to the solid-not-fat in the different portions of a milking, it may be stated without fear of contradiction that there is no appreciable variation. These constituents of milk remain remarkably constant in all portions of the milk throughout the milking. Breeds may vary in regard to their average solids-not-fat percentage, but this fact has no bearing upon the distribution of these constituents in the mammary gland.

By studying Table IV it is noticed that there is apparently no marked correlation between the percentage of fat and the rate of milk flow. The relatively high correlation coefficient secured in some instances loses significance when correlation coefficients on other data secured under similar conditions show no relationship between the rate of milk flow and fat percentage.

Conclusions.

(1) The milk in the teat canal and milk cisterns is present in such a state as to allow creaming to proceed.

(2) The larger fat globules have difficulty in passing down the narrow milk ducts and are only removed with the last-drawn milk.

(3) The milk which has been in the reservoir for some time has not had part of its contained fat reabsorbed by the lymphatics.

(4) Fat secretion is not stimulated by the milking process.

(5) Individuals vary widely with regard to udder and individual deposition, making studies on milk secretion very difficult.

(6) The percentages of solids-not-fat in different fractions of a milking, are constant.

(7) There is no correlation between the rate of milk flow and the fat-percentage.

Acknowledgments.

I am indebted to Professor Earl Weaver, now of the Oklakoma A and M, who made this work possible. I also wish to acknowledge the valuable technical assistance of C. A. Matthews, Department of Dairy Industry, Washington, D.C.

Section X.

Miscellaneous.

H. H. Curson ...	Anatomical Studies No. 19: Polydactyly in a Springbok and a Pig.
H. H. Curson and N. T. v. d. Linde	Anatomical Studies No. 20: Hernia of the Brain in an Afrikander Heifer.
H. H. Curson ...	Anatomical Studies No. 23: Persistence of Urachus in a Yearling Bull.
D. Sigwart and H. H. Curson	Anatomical Studies No. 24: Amniotic Adhesion in a Calf.
H. H. Curson ...	Anatomical Studies No. 25: Ankyloblepharon, Monophthalmia, Aplasia *N. optici* and Hypoplasia Cerebri in a Kid.
C. Jackson	Anatomical Studies No. 26: Bilateral Double Ureter and Congenital Hydronephrosis in a Pig.
C. Jackson	Anatomical Studies No. 27: An Anomaly of the Flexor Digitorum Pedis Profundus in a Goat.
H. H. Curson ...	An Unusual Foreign Body from a Jackal.
H. H. Curson and J. Quinlan	Anchylosis of the Hock Joint in a Steenbok.

Anatomical Studies, No. 19: Polydactyly in a Springbok and a Pig.

By Dr. H. H. CURSON, Veterinary Research Officer, Onderstepoort.

The above condition while apparently uncommon in wild mammals, is undoubtedly far from rare in domesticated animals.

Case 1.—The springbok material was found in the Anatomical Museum on taking charge in 1927, but, unfortunately, no details are available except that we are indebted to Dr. E. C. N. van Hoepen, Director, National Museum, Bloemfontein, for its despatch. The photograph (see Fig. 1), however, clearly demonstrates the nature of the anomaly. It would appear that the first and third specimens of the group form a pair and belonged either to the pectoral or pelvic limb, and that the second or middle " foot " belonged to one of the two limbs forming the opposite pair. In other words suppose the first and third " feet " were pelvic, then the second " foot " of the group belonged to one of the pectoral limbs. As only one pair of the two (second specimen) is well developed and as this would most likely be situated medially, it would appear that the second " foot " belonged to a left limb. If this view be correct then the first and third " feet " belonged to right and left limbs respectively. In the first specimen there were present four chief digits (two being normally developed) and two vestigial digits situated behind the fetlock joint. The middle or second specimen presented also four main digits, while posteriorly only one vestigial digit was to be observed. The third " foot " or specimen showed three chief digits anteriorly while behind the fetlock joint a vestigial digit was present in each case.

Case 2.—The pig's feet (both fore) were despatched to this Institution on 16/6/30 by Government Veterinary Officer Chalmers (Johannesburg), to whom they had been presented by Mr. W. Midley, Superintendent, Germiston Abattoir (File 126/11). Our thanks are due to both these gentlemen for their interest in the Teratological Museum. See Figs. 2 and 3 (Sp. 1596).

The left " foot " comprised not only five metacarpal bones, but also five digits, the 3rd and 4th as usual, being the major digits, and the 2nd and 5th the minor. In addition there was present the first metacarpal bone with a digit slightly larger than the 2nd. The right " foot," however, showed only four metacarpal bones, these being assumed to be the 2nd-5th. They were, as in the left " foot " five digits, the major being the 3rd and 4th. With the median metacarpal bone (2nd) articulated the largest of the three minor digits, and with the 5th metacarpal the next digit in size was to be found.

The smallest possessed no articulation with any of the metacarpal bones, but was placed between the 1st and 3rd digits, apparently in more intimate (fibrous) relation with the 1st digit.

Owing to the abundance of anatomical material, etc., the dissection of the soft structures could not be undertaken.

As to the causation of polydactylism, whereas formerly it was customary to ascribe the condition either to atavism (Case 2) or to dichotomy (Case 1), Stockard (1921) indicates that all anomalies might be attributed to any interference in utero with rate of development.

REFERENCE.

STOCKARD, C. R. (1921). "Development Rate and Structural Expression, etc." *American Jnl. Anat.*, Vol. 28, pp. 116-263.

FIG. 1.

FIG. 2.

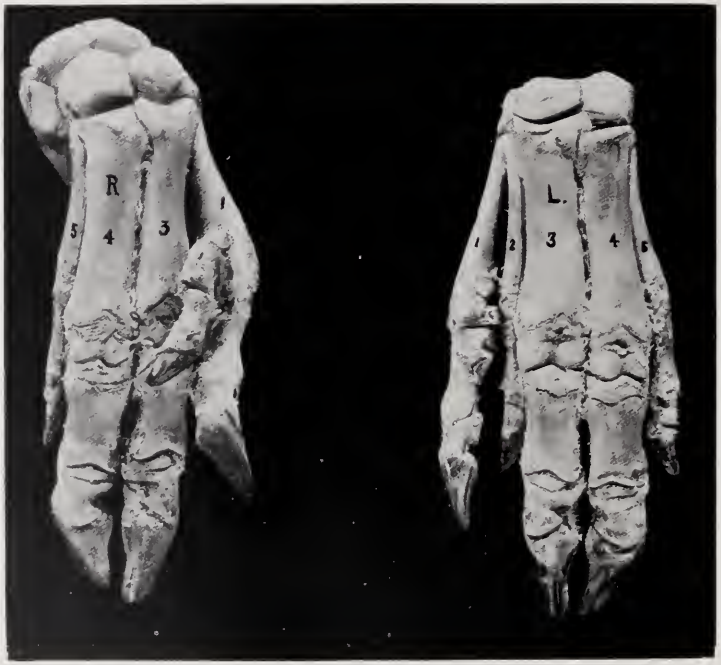

FIG. 3.

17th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1931.

Anatomical Studies, No. 20: Hernia of the Brain in an Afrikander Heifer.

By Dr. H. H. CURSON and N. T. v.d. LINDE, Faculty of Veterinary Science, University of Pretoria, South Africa.

Cephalocele or cerebral hernia, while not infrequent in young monsters, which usually die soon after birth, is certainly of rare occurrence in otherwise normal adult cattle, the subject of this note being an Afrikander heifer which died as the result of an accident at the age of approximately 4½ years.

At birth a large round swelling, beneath the skin in the region of the opening shown in the attached photograph (see figure), was observed. On pressure there was definite evidence of pain, but the animal progressed satisfactorily and was always in good condition.

After death, a dissection of the area in question revealed the following: The rim of the more or less circular opening, averaging 4 cm. in diameter, was strongly developed and irregularly elevated except at the ventral aspect, where there was a depression. The smaller opening (shown in the photograph) towards the right side of the lower part of the rim is artificial and was due to an accidental fracture of the bone. Situated in the cavity beneath the skin was fatty tissue, beneath which the dura matter covering the frontal poles of the cerebrum was observed.

The skull, labelled T. 18 (Path. Specimen 9,292), has been placed in the Teratological Museum.

17th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1931.

Anatomical Studies, No. 23*: Persistence of Urachus in a Yearling Bull.

By Dr. H. H. CURSON, F.R.C.V.S., Veterinary Research Officer, Onderstepoort.

A yearling bull calf (Fries), numbered 3704 was killed for experimental purposes on 15/8/30 (P.M. 9594). On post-mortem examination it was observed that the urinary bladder was anchored to the umbilical region, but before describing the condition in detail, it seems necessary to recapitulate the changes, associated with the bladder, which occur at birth.

As a result of rupture of the umbilical cord, blood no longer flows through the umbilical vessels and the umbilical arteries become the round ligaments of the bladder. The distal portion of the allantoic stalk or urachus (i.e. from the bladder to the umbilicus) is transformed into a fibrous cord, the middle ligament of the bladder, which is represented on the vertex as a scar (centrum verticis). As a consequence, the bladder is fixed chiefly at its neck while the receptacle itself is more or less freely movable depending on the amount of urine it contains. (See figure.)

In the case in question the following anomalies were encountered:—

(*a*) The bladder was an elongated tube approximately 35 cm. in length and 6 cm. at its widest part, but the round ligaments were normal in situation.

(*b*) The distal portion of the urachus, instead of atrophying, had persisted, indeed had become thickened. On opening this passage, approximately 22 by 2 cm., it was found to be filled with pus.

(*c*) The umbilicus was covered by dried pus which concealed a minute opening into the urachus. Pressure, however, along (*b*) resulted in a small quantity of creamy pus escaping from the orifice which should have closed after birth.

As a result of the above changes the bladder was anchored to the umbilical region and hence remained tube-like in form.

* Studies 21 and 22 were published in the *J. S.A.V.M.A.*, Vol. 1, No. 4 (1930).

Obviously the calf had suffered from navel-ill, but, fortunately, upward extension of the pus infection had become arrested at the point where the round ligaments were attached to the bladder and the bladder epithelium was quite intact.

Cases of urinary fistula in domesticated animals, i.e. where the vertex of the bladder remains patent and opens upon the external surface (pervious urachus) are not infrequent, but closure of the bladder associated with persistence of the distal portion of the urachus is uncommon.

Also affecting the calf was an epidermoid cyst (Curson and Thomas, 1930) 1.5 cm. in diameter and situated 3 cm. from the middle line of the lower aspect of the neck. The structure was placed superficially to the Momo-hyoideus approximately 6 cm. from its insertion. The wall was tough and 0.3 cm. thick and surrounded by the fat of the subcutaneous region. The contents were a dirty brown pasty material (sebaceous in origin) and dark brown to black hairs which were either projecting from the inner wall or shed. In the latter case they were arranged in nest-like fashion.

I am indebted to Mr. P. L. le Roux, B.Sc., M.R.C.V.S., for the specimens, both of which have been placed in the Teratological collection (Path: 10612).

REFERENCE.

CURSON, H. H., and THOMAS, A. D. (1930). Anatomical Studies, No. 17: Epidermoid Cysts in Domesticated Animals. *16th Rept. of Dir. Vety. Services and Animal Industry*, pp. 585-588.

Anatomical Studies, No. 24: Amniotic Adhesion in a Calf.

By Dr. H. SIGWART, Keetmanshoop, and Dr. H. H. CURSON, Veterinary Research Officer, Onderstepoort.

Amniotic or amniogenous adhesions are rare in domesticated animals, in fact, the above case being the first encountered by the authors in an experience covering many years. Joest, however, has ably described the condition and its sequelae (1921, 1922).

The specimen in question, the head of a foetus of approximately 8-9 months, was sent to the Teratological Museum, Veterinary Research Laboratories, by the first-named author, who wrote on 18/2/30 (Vet. Res. Lab. File 255/37) as follows: ". . . the amnion had a connection with the head and brain of the calf and the foetus was therefore announced to me as having a second navel cord. . . . My examination could prove that the amnion and with it blood vessels were firmly attached to the vertex of the . . . head, and the navel-like cord penetrated the one os frontale and was in direct . . . contact with the cerebrum. Unfortunately, by dissecting the head . . . the soft cerebral tissue slipped off . . ." Photos (see Figs. A and B) taken at the time illustrate very clearly the nature of the anomaly (Path. No. 10,075).

Accompanying the head of the foetus was that portion of the foetal membranes showing the navel-like cord. The brain had been removed from the cranial cavity and the roof and lateral walls had been disturbed, but through the floor of the cranium, on the left side immediately posterior to the orbital wing of the os sphenoidale, there was a strong fibrous cord (about 2.5 cm.) which was firmly attached to the periorbital sheath in the vicinity of the apex.

If less than the normal amount of amniotic fluid is present, then amniotic attachment to the foetus may occur.

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JOEST, E. (1921). *Spezielle pathologische Anatomie der Haustiere*, Vol. 2, p. 478. Richard Schoetz, Berlin.

JOEST, E. (1922). Über amniogene Missbildungen bei Haustieren. *Berliner Tierärz. Wochen.*, Jahrg. 38, No. 33, pp. 381-384.

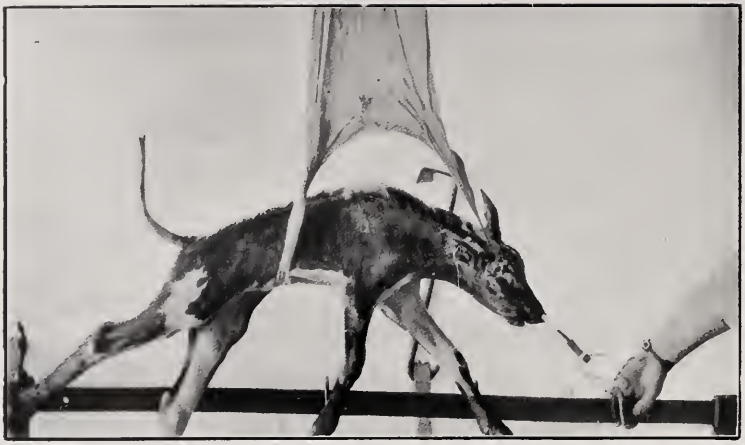


FIG. A.

FIG. B.

Anatomical Studies, No. 25: Ankyloblepharon, Monophthalmia, Aplasia N. Optici, and Hypoplasia Cerebri in a Kid.

By Dr. H. H. CURSON, F.R.C.V.S., Veterinary Research Officer, Onderstepoort.

A male kid, born 28/12/29 at Onderstepoort, and showing deformities of the eyelids, was sent to the Anatomical Department for examination on 5/2/30 when it was killed.

On inspection it was found that there was partial ankyloblepharon especially of the left eyelids, this condition being well shown in Figs. 1 and 2. Dissection showed that the left eyeball was missing. The brain was thereupon examined and it was found that not only was there aplasia nervi optici but also hypoplasia of the left cerebral hemisphere. For comparison the brain of a normal but older kid was dissected and both specimens are shown in Fig. 3. Note the differences in the arrangement of the gyri and sulci.

Anophthalmia congenita has been described by Kitt (1927) in the horse, calf, ox and fowl, but Joest (1921) refers to the condition in the sheep as well. The above represents the only case observed by the author in a kid.

Obviously the monophthalmia is due to an arrest in the development of the optic evaginations from the prosencephalon during early embryonic life.

The above specimens have been placed in the Teratological collection under the Path. No. 10,313.

REFERENCES.

JOEST, E. (1921). *Spezielle pathologische Anatomie der Haustiere*, Vol. 2, p. 177. R. Schoetz, Berlin.

KITT, T. (1927). *Pathologische Anatomie der Haustiere*, Vol. 3, p. 594. F. Enke, Stuttgart.

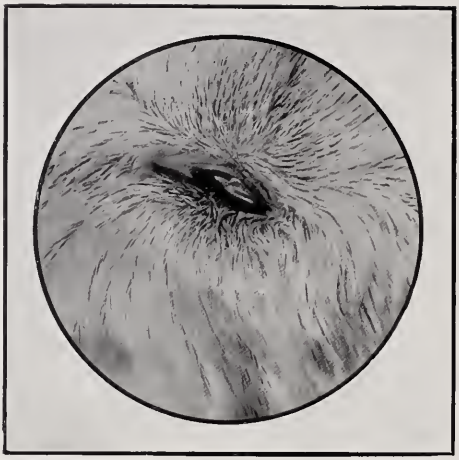


FIG. 1.—Left Eye.

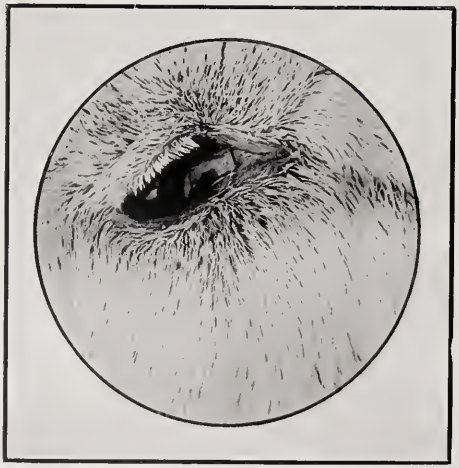


FIG. 2.—Right Eye.

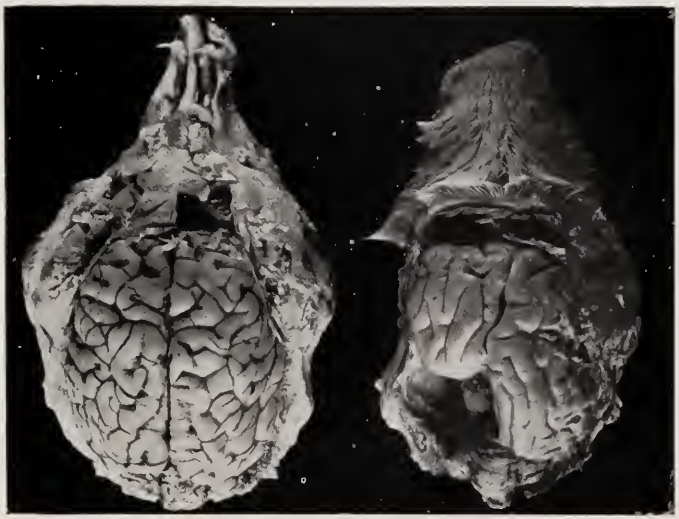


FIG. 3.

17th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1931.

Anatomical Studies No. 26*: Bilateral Double Ureter and Congenital Hydronephrosis in a Pig.

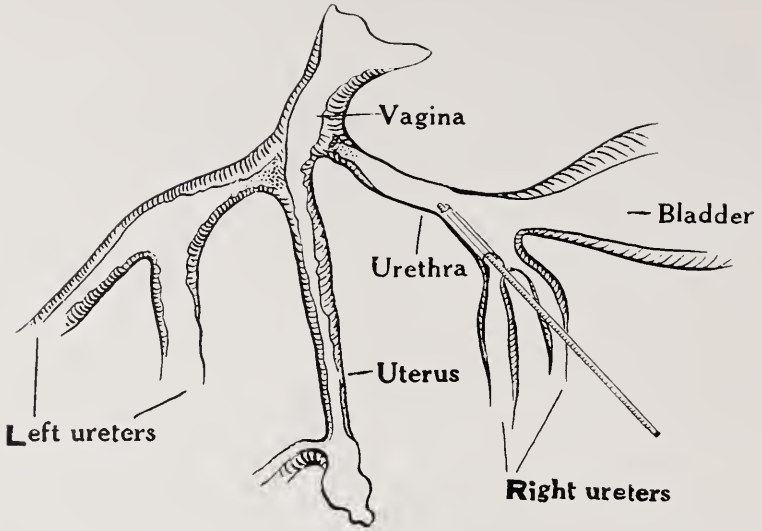
By CECIL JACKSON, B.Sc., B.V.Sc., Veterinary Research Officer, Onderstepoort.

It is well known that among domestic animals the pig is the commonest subject of hydronephrosis. This is considered to be due to the anatomical relationships of the ureter and urinary bladder. This explanation cannot, of course, be cited in cases of congenital hydronephrosis, and it is curious that this condition also has been recorded most often in the pig, and still more so that double ureter appears to occur most frequently in the same animal.

This short note places on record a case of malformation of the urinary system which, combining as it does the following abnormalities, is of unusual interest: bilateral complete(?) double ureter and pelvis; ureters of the one side connected with the vagina, with atresia of the orifice; ureters of the other side connected with the urethra, with atresia of the vaginal orifice of the urethra; and associated hydronephrosis.

The subject—a female piglet (Path. No. 10400), which died from uraemia, and which Mr. W. O. Neitz, B.V.Sc., of this Institute, was kind enough to bring to my notice, survived for about two days after birth. The text figure may be amplified by the following observations: The kidneys are much enlarged, fluctuating, and rather thin-walled sacs. Each is divided by the dilated calyces into a number of compartments filled with urine, and no communication exists between the two pelves. From each of the latter leads a ureter, greatly dilated with urine on the left side, much less so on the right. The urine stasis is at first sight referable to the peculiar mode of termination of the ureters. On the right, they open independently into the urethra at its emergence from the neck of the bladder—i.e. a complete double ureter exists on this side. The vaginal orifice of the urethra is atretic. On the left side the ureters join shortly before their termination, and the common duct so formed leads to the vagina at a point opposite the urethral termination. Here again, the lumen disappears as the wall of the vagina is reached. Both pairs of ureters are remarkably sinuous and a marked degree of twisting is present, although (from the circumstance that they can be completely unravelled without resorting to transection) it is seen that no actual plaiting has taken place. The bladder is not markedly distended with urine and the urachus is closed.

* Cases of interest encountered in the Anatomical Department of the Faculty of Veterinary Science (University of Pretoria) are published under this title.



Termination of the Ureters (Piglet, *Path.* No. 10, 400).

Histology.

In the wall of the sacs which constitute the kidneys, all the layers can be distinguished, i.e. cortex, medulla and limiting zones are distinct, but marked atrophy has taken place and numerous tubules are distended, many containing cellular casts. An appreciable degree of fibrosis is present, especially in the flattened papillae. The ureters are lined by the usual transitional epithelium as also is the duct formed by the union of the ureters of the left side.

Embryological Discussion.

Several points emerging from these facts deserve comment:—

1. The malformation of double ureter is, of course, referable to anomalous development of the pelvic anlage from the mesonephric duct, i.e. duplication of the kidney bud. Cases of double ureter may be held to afford evidence in support of the now widely accepted doctrine of the dual origin of the kidney parenchyma. The two sets of sprouts (forming the ducts of Bellini and the collecting tubules) which spring from the terminal expansions of the double ureteric evagination become invested with a common mass of secretory tubules by the nephrogenic tissue (mesoderm).

2. The termination of ureters in the urethra is explicable on the simple ground of the failure of the orifices (which originally are connected with the caudal end of the Wolffian duct) to migrate farther than the urogenital sinus, which later becomes the female urethra.

3. In accounting for termination of a ureter in the vagina, more extensive considerations are involved. Before dealing with these, it is well briefly to recall the existing theories of the origin of the Müllerian ducts in higher forms. These are two in number:

(i) The Müllerian duct arises by invagination of the mesothelium of the body cavity and then grows caudally by proliferation of its own cells. The process is entirely independent of the Wolffian duct.

(ii) The cranial portion of the duct having arisen as described above, the caudal portion is formed by longitudinal fission of the Wolffian duct, just as occurs in the lower vertebrates.

An explanation of ureters opening into the vagina, which presumably is based on the "proliferative" theory (i), is that the ureters fail to migrate cranially along the wall of the urogenital sinus, and as the caudal ends of the fused Müllerian ducts enlarge to form the uterus and vagina, the ureteral openings become incorporated in their walls.

A far simpler interpretation could, however, be based on the "fission" theory (ii), whereby the ureters (originally connected with the caudal extremities of the Wolffian ducts and failing to be incorporated in the wall of the urogenital sinus when the latter becomes separated from the rectum by the uro-rectal fold) are taken up into the wall of the Müllerian duct as the latter splits off from the Wolffian duct. This whole problem has, in the present case, tended considerably to obscure the issue to the question (discussed below) as to whether or not the ureter of the left side is to be regarded as completely double.

4. The question arises as to what are the criteria of complete double ureter. Where double ureters open into the bladder, the position is simple. In the present case also, it is clear that one is justified in speaking of a complete double ureter on the right side (where the ureters terminate in the urethra). On the left side, however, the position becomes more complicated. Is one debarred, because the ureters join before their termination, from claiming completeness for their duplicity, although they are as long as, if not longer than, those of the right side? The difficulty might be circumvented by regarding the common duct produced by the ureteral junction as a urethra, but this view is not permissible since—

(*a*) anatomically the duct in question does not mediate between the bladder and the vagina;

(*b*) histologically it is lined by transitional and not by stratified epithelium; and

(*c*) embryologically the interpretation is strained for reasons which are self apparent.

It seems only reasonable to conclude that the one satisfactory criterion of completeness in cases of double ureter is that the question "Did the malformation arise by duplicature and not by bifurcation of the kidney evagination?" be answerable in the affirmative. Judging by this standard one has little hesitation in deciding that

branching of a single bud took place, and consequently it is concluded that it would be erroneous to consider the double ureter of the left side as being complete. The malformation described in the second paragraph of this article must, therefore, be amended, as suggested by the mark of interrogation, to read " bilateral double ureter, one complete and one partial."

Pathogenesis.

It remains to discuss the actual pathogenesis of the urinary stasis which was present. From the facts as set out above, it will readily be appreciated that an inadequacy of the excretory path on the right side due to the atretic vaginal orifice of the urethra could not have become operative in producing hydronephrosis until after the closure of the urachus; while on the left side, a stasis due to atresia of the vaginal orifice of the ureters would have existed from the time of commencement of secretion by the permanent kidney. Such an interpretation derives support from the inequality of the ureteral dilatation to which reference has already been made.

On the other hand, the hydronephrosis was almost as marked on the right side as on the left, and it is hard to believe that the changes in the right kidney could have occurred within a space of two days. Further, it seems improbable that the urachus would in fact have closed against the pressure of such a urine stasis. Finally, the absence of a marked distension of the bladder speaks rather conclusively against this view.

It seems probable, therefore, that the atresia was at least not the sole obstructive factor, and in this connection the remarkable intertwining of the ureters appears to be of aetiological significance, more especially since this twisting would be likely to produce the intermittant type of interference which typically is so favourable to the production of marked hydronephrosis. It will be remembered that the ureters of the pig are normally slightly flexuous, a disposition which in this subject was probably exaggerated originally and doubtless became still more so on account of the dilatation of the double ureters.

It is realised that the features of this case, taken individually, are already to be found in the literature. Nevertheless, it has appeared desirable that such an unusual *combination* of abnormalities should be recorded, at the risk of some repetition. The case further serves to illustrate the well-known though rather surprising fact of the difficulty of assigning with certainty a cause to many cases of hydronephrosis.

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Anatomical Studies No. 27*: An Anomaly of the *Flexor Digitorum Pedis Profundus* in a Goat.

By C. JACKSON, B.Sc., B.V.Sc., Veterinary Research Officer, Onderstepoort.

The deep flexor of the hind limb in ruminants is (apart from its insertion) very similar to the same muscle in the horse, except for variations in the relative size of its three components. These differences, mentioned by Sisson (1917), appear to be common to both the small and the large ruminants.

Normally the tendon of the *flexor hallucis longus* (deep head) is joined by that of the *tibialis posterior* to constitute the main tendon of the deep flexor. This junction in ruminants takes place on a level with the medial malleolus of the tibia.† The *flexor digitorum longus* (medial head) joins the main tendon on a level with the head of the metacarpus (not at the junction of the middle and proximal thirds of the metacarpus, as in the horse).†

In the right hind limb of the goat in question (a dissection subject—D.O.B. No. 18637) the *tibialis posterior* terminated in the upper third of the crural region on two tendons. Of these, the much larger (medial) one joined the tendon of the *flexor digitorum longus* at about the level of the *tuber calcis*, while the smaller (posterior) one joined tendon on the *flexor hallucis longus* at about the junction of the middle and distal thirds of the tibia. The combined tendon of the *flexor digitorum longus* and the *tibialis posterior* descended the canal in the medial ligament of the hock and joined the tendon of the *flexor hallucis longus* in the usual manner.

The arrangement is clearly seen in the accompanying text figure in which the normal (left) is contrasted with the abnormal (right) limb.

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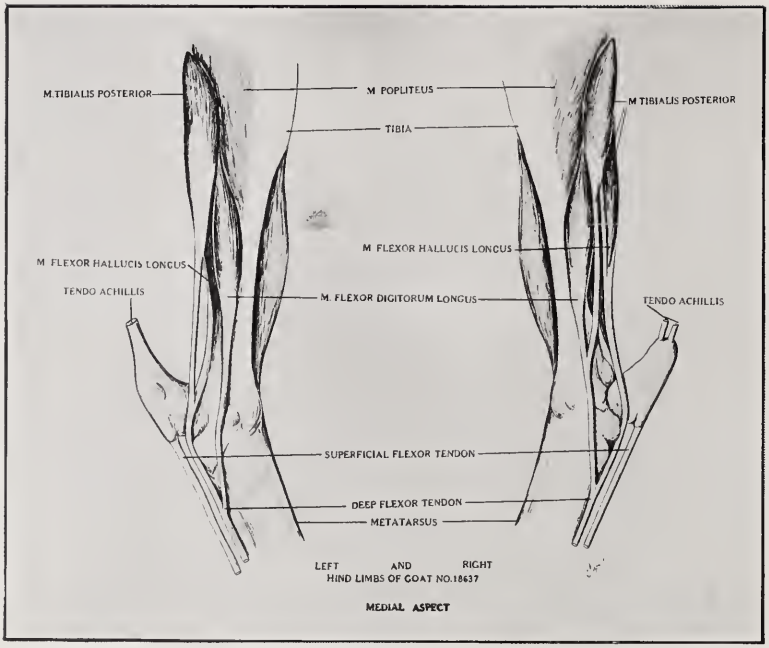
* Cases of interest encountered in the Anatomical Department of the Faculty of Veterinary Science (University of Pretoria) are published under this title.

† This is one of a number of points in comparative myology, observed from time to time in the Department, but unrecorded in the available literature, and which it is hoped to publish in a further communication.


M. TIBIALIS POSTERIOR
M. POPLITEUS
TIBIA
M. TIBIALIS POSTERIOR
M. FLEXOR HALLUCIS LONGUS
M. FLEXOR HALLUCIS LONGUS
TENDO ACHILLIS
M. FLEXOR DIGITORUM LONGUS
TENDO ACHILLIS
SUPERFICIAL FLEXOR TENDON
DEEP FLEXOR TENDON
METATARSUS
LEFT AND RIGHT
HIND LIMBS OF GOAT NO. 18637
MEDIAL ASPECT

17th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1931.

An Unusual Foreign Body from a Jackal.

By Dr. H. H. CURSON, F.R.C.V.S., Veterinary Research Officer, Onderstepoort.

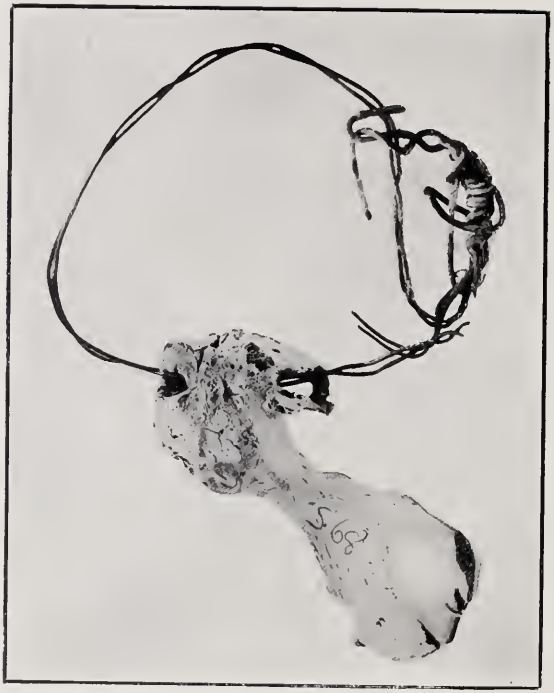
On the 26th June, 1930, was received from Dr. van Hoepen, Director, National Museum, Bloemfontein, the left os coxae of a jackal which apparently had died as result of the wire loop which had become attached to the bone in question (see figure). Judging from the weather beaten appearance of the bone and wire it would appear that death on the veld had taken place a considerable time previously although a few hairs were to be seen in the knotted wire. (Lab. Rpt. Sp. 2002 and File 141/19).

At an average distance of 4.5 cm. from the upper border of the acetabulum a double thickness of thin soft wire appeared to have penetrated the os ilium, in fact, had become embedded in an oblique direction immediately anterior to the facies auricularis. Presumably the wire, on reaching the os ilium, set up a suppurative ostitis as a result of which callus formation occurred around the foreign object, thus leading to the formation of a channel 5 cm. in length in which was situated the wire. In consequence the crista iliaca, tuber sacrale and tuber coxae were no longer evident.

It would seem that a noose trap had been set, probably at the entrance to the burrow of a jackal, and the unfortunate animal, instead of being caught at the neck as was intended, managed to get through the loop as far as the hindquarters which became strangulated. As a result of the terrific struggle which undoubtedly followed, the wire attachment broke, but in such a manner that the victim could not escape from the loop. In the course of time the strangulating wire, cutting through the superficial structures, became incorporated in the callus growth from the os ilium as described above.

Dr. Quinlan considers that such a lesion would have taken approximately three months to form.

Thanks are due to Dr. van Hoepen for sending a specimen of such interest to this Institution (Museum S.68).



Anchylosis of the Hock Joint in a Steenbok.

By Drs. H. H. CURSON and J. QUINLAN, Veterinary Research Officers, Onderstepoort.

In July, 1928, Mr. W. G. Barnard, of the Transvaal University College, Pretoria, while in the Lydenburg District, shot a steenbok (*Pediotragus* sp.) which he noticed to be running with difficulty.

On inspecting the dead animal only the left limb was noticed to be abnormal (see figure), but in view of the lesions to be described, it seems extraordinary that the animal was able to avoid the hunter so long.

The tibia (distal extremity) was approximately double the normal size in width and its articular surface was distorted, forming a false joint with the distally situated tarsal bones, some of which formed an irregular bony mass. The bones so involved were the os tarsi fibulare, os tarsi tibiale, and the os centro-tarsale quartum, while the os maleolare was fused to the tibial bone. Articulating normally with the above described irregular bony structure were the large metatarsal, 2nd and 3rd tarsal, and 1st tarsal bones. Also associated with the hock joint were several irregular bony splinters which were attached by fibrous tissue to the medial, posterior and lateral aspects.

As can be imagined not only was there limited movement at the hock joint, but the distal articulations were also involved, being in a state of flexion, so that the flexor (back) tendons had become contracted. As a result of this contraction the foot became deformed, the wall in the region of the "toes" being worn away, and the heels being abnormally high. In fact the foot had assumed the shape of a typical boxy foot.

The cause of the condition was most likely a fracture involving the tibia and neighbouring bones due to a bullet wound.

Mr. Barnard states that he subsequently noticed a similar lesion in a duiker (*Sylvicapra grimmi*).

ANCHYLOSIS OF THE HOCK JOINT IN A STEENBOK.